High moisture extrusion of plant proteins

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Zusammenfassung

Das Hauptziel der Dissertation war die Entwicklung und Bewertung von Fleischanaloga aus alternativen Proteinquellen wie Soja, Lupine und Mikroalgen mittels der Nassextrusionstechnologie.

Im ersten Teil der Arbeit wurde der Einfluss von Extrusionsbedingungen wie Extrusionstemperatur (135–180 °C), Wassergehalt (40–68 %) und Schneckendrehzahl (400–1800 U / min) auf das Extruderverhalten und die Eigenschaften der Extrudate untersucht. Die Fleischanaloga wurden auf Basis von Lupinenproteinkonzentrat und -isolat (50:50) produziert. Die Extrudertemperatur zeigte einen geringen Einfluss auf die getesteten Eigenschaften. Der Wassergehalt zeigte eine negative lineare Korrelation bei allen Extruderparametern, beim Farbunterschied und der Schnittfestigkeit von Extrudaten. Die Produkttemperatur am Extruder, die spezifische mechanische Energie und die Kochausbeute der Extrudate waren mit zunehmender Schneckendrehzahl erhöht. Eine erhöhte *in-vitro*-Verdaulichkeit wurde bei höherem Wassergehalt gefunden. Fleischanaloga zeigten eine bessere Texturierung bei erhöhter Temperatur und Schneckendrehzahl zusammen mit einem verringerten Wassergehalt.

Um die Nutzbarkeit der *Spirulina*-Biomasse für die Nassextrusion zu prüfen, wurden die Versuche mit der Lupinenproteinmischung durchgeführt. Der Einfluss verschiedener Spirulinagehalt (bis zu 50 % Trockenmasse) und von Extrudergehäusetemperatur (145 ° C, 160 ° C und 175 ° C), Wassergehalt (50, 55 und 60 %) und Schneckendrehzahl (500, 800 und 1200 U / min) auf die Extrudateigenschaften wurde untersucht. Die physikalischen Eigenschaften wie Textur, Kochausbeute und Wasserhaltekapazität wurden durch den Spirulinagehalt und die Extrusionsparameter signifikant beeinflusst. Erhöhte Temperatur und Schneckendrehzahl sowie verringerter Wassergehalt verbesserten den Anteil von Gesamtflavonoiden, Gesamtphenol- und Antioxidansaktivität von Fleischanaloga geringfügig, und die Zugabe von *Spirulina*-Biomasse erhöhte diese Eigenschaften signifikant. Die Zugabe von *Spirulina* beeinflusste die *in-vitro*-Proteinverdaulichkeit der Extrudate negativ. Dieser Effekt wurde durch Erhöhung von Wassergehalt und Schneckendrehzahl ausgeglichen. Darüber hinaus veränderte das Extrusionsverfahren die sekundären strukturellen Eigenschaften der Proteine in den Fleischanaloga.

Weiterhin wurde die Anwendung von Iota (ι) Carrageenan (ICGN) als Additiv für Sojafleischanaloga mittels Planetenwalzenextruders untersucht. Die Zugabe von ICGN erhöhte die Schnittfestigkeit und Elastizität signifikant und verringerte die Wasserhaltekapazität und die Kochausbeute. Zusätzlich ergab die Zugabe von ICGN eine kompakte Netzwerkstruktur. Die sensorische Bewertung ergab, dass das Fleischanalogon mit 1,5 % ICGN am besten akzeptiert wurde und die Zugabe von ICGN die Faserstruktur / Texturierung, eine Schlüsseleigenschaft der Fleischanaloga, verbesserte.

Schlagworte: Nassextrusion; pflanzliche Proteine; Fleischanaloga

Abstract

The main aim of the thesis was to develop and evaluate the meat analogues from alternative protein sources such as soya, lupin and microalgae using high moisture extrusion (HME) processing.

In the first part of the work, the effect of extrusion conditions, such as barrel temperature (135–180 °C), water feed (40–68 %) and screw speed (400–1800 rpm) on extruder responses and properties of the meat analogues based on lupin protein concentrate and isolate (50:50) was investigated. Barrel temperature showed negligible influence on the tested properties. A negative linear relationship of water feed was found on all of the extruder responses and on colour difference and cutting force of extrudates. Product temperature at extruder, specific mechanical energy and cooking yield of the extrudates were increased with increased screw speed. An increased *in-vitro* protein digestibility (IVPD) was observed with higher water feed content. Meat analogues showed better texturization with increased temperature and screw speed along with decreased water content.

The investigation further continued with the aim of testing the feasibility of incorporating *Spirulina platensis* biomass with lupin protein mixtures for HME. The influence of different *Spirulina* concentrations (up to 50 % by dry mass), extruder barrel temperature (145 °C, 160 °C and 175 °C), water feed (50, 55 and 60 %), and screw speed (500, 800 and 1200 rpm) on the extrudate properties was investigated. The physical properties, such as texture, cooking yield and expressible moisture were significantly affected by the *Spirulina* addition and extrusion parameters. Increased temperature and screw speed as well as decreased water feed slightly improved the content of total flavonoids, total phenolics and antioxidant activity of meat analogues, and the addition of *Spirulina* biomass significantly increased those properties. The addition of *Spirulina* negatively influenced the IVPD of extrudates, however, this effect was counterbalanced with the controlled extrusion conditions, such as increased water feed and screw speed. Furthermore, the extrusion process modified the secondary structural properties of the proteins in the meat analogues.

Further, the application of iota (ι) carrageenan (ICGN) as an additive was investigated to develop a soya meat analogue using a planetary roller extruder (PRE). The addition of ICGN significantly increased the cutting force and elasticity and decreased expressible moisture and cooking yield. Additionally, an increase of the compact network structure of the extrudates was observed with the addition of ICGN by the scanning electron microscopy. Sensory evaluation revealed that the meat analogue with 1.5 % ICGN was best accepted, and the addition of ICGN improved the fibrous structure/texturization, which is a key property of the meat analogues.
Keywords: High moisture extrusion; plant proteins; meat analogues

Preface

The thesis work has been published in the following peer reviewed publications:

- Palanisamy M, Franke K, Berger RG, Heinz V, Töpfl S (2019) High moisture extrusion of lupin protein: influence of extrusion parameters on extruder responses and product properties. J Sci Food Agric. 99: 2175–2185. doi: 10.1002/jsfa.9410
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- Palanisamy M, Töpfl S, Aganovic K, Berger RG (2018) Influence of iota carrageenan addition on the properties of soya protein meat analogues. LWT - Food Sci Technol 87:546–552. doi: 10.1016/j.lwt.2017.09.029

Contents

Abbreviations

ANOVA	One way analysis of variance
CO2e	Carbon dioxide equivalents
FAO	Food and Agriculture Organization
FTIR	Fourier-transform infrared spectroscopy
HME	High moisture extrusion
ICGN	Iota carrageenan
IVPD	*In-vitro* protein digestibility
PRE	Planetary roller extruder
SEM	Scanning electron microscopy
SME	Specific mechanical energy
Sp.	Species
TEAC	Trolox equivalent antioxidant activity
TFC	Total flavonoid content
TPC	Total phenolic content
WHC	Water holding capacity

1. Critical evaluation of research topic and most important results to the scientific state-of-the-art

1.1. World population and future protein demand

Due to the forecasted increase in world population in the next two to three decades, concerns over producing adequate food protein for the projected 10 billion people by 2050 are inevitable. It is projected that, compared to 2006, the world would need 70 % more food in order to meet the demand in 2050. In the 17 sustainable goals adopted by the United Nations, "ensuring global food security" stands in second place in the agenda for 2030 on sustainable development [1]. It is implausible that only increasing the agricultural production efficiency will solve this food gap problem. The major source of protein is meat especially in developed countries for the production of which enormous amounts of resources are spent. Almost 30 % of global greenhouse gas emissions arise from food production [2], from which meat production accounts for 14.5 % [3].

Hence, both the agricultural productivity improvement and changing the diet pattern from animal based foods to plant based could mitigate the increasing food demand in future, as the plants based diet requires less resources and has less environmental impact than an animal based diet [4]. For the production of livestock in Europe, more than 80 % of the protein is imported from outside of Europe which originates mostly not from environmental-friendly sources [5]. For one kg of edible protein generation from beef, 282.6 m^2 land, 109 m^3 water, 2.7 liters of fuel (includes gasoline and diesel used on the farm for agricultural and livestock production), 1.94 kg fertilizer (includes N, P and K) and 93 g pesticide (used for raising animals and for growing animal feed) are required. Mejia et al reported from life cycle assessments that one kg of beef protein production generated 45–640 kg of carbon dioxide equivalents (CO_2e), whereas 10 kg CO_2e per kg of protein is produced from tofu. Other meats such as pork, chicken, and seafood (fisheries) score with ranges of 20–55, 10–30, and 4–540 kg CO_2e per kg of protein, respectively [6].

Besides the higher meat consumption in developed countries, due to the improving economical situation, the developing countries are also having a shift towards westernized diet, which contains more meat and is higher in saturated fat, sugar, and refined foods and lower in fiber [7].

Comparing to the predicted diet patterns in 2050, a shift towards a complete vegetarian diet worldwide could lead to 55 % per capita reduction in emissions occurring from food production [8]. For example in Germany, switching from the omnivore diet to an ovo-lacto-vegetarian could result in a one third reduction of emissions and even up to 50 % less emissions in case of a vegan diet [9]. Therefore, the question of how to achieve the change in eating behavior to reduce the meat consumption is gaining attention among scientists and social workers.

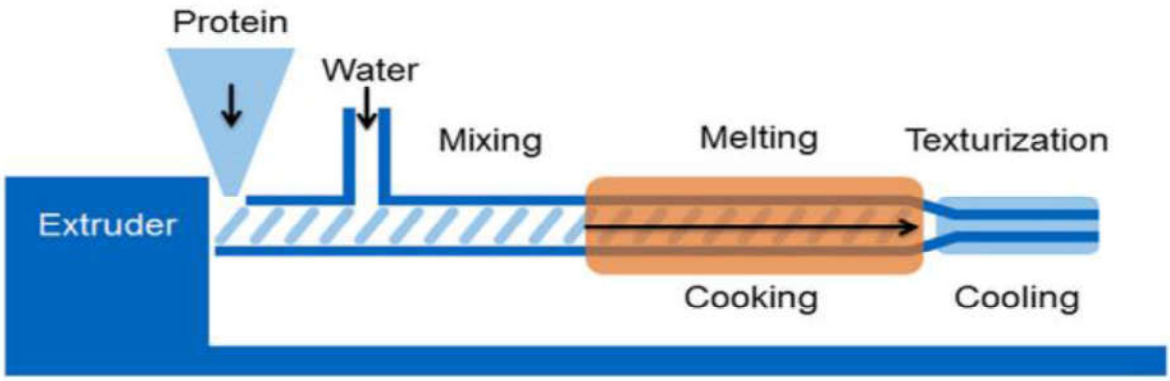
One of the strategies that can be used to reduce the meat consumption is developing meat analogue products from sustainable alternative protein sources [10].

1.2. Plant-based meat analogues

Meat analogues are referred to as foods which have texture, mouth-feeling, taste, and nutritional value similar to meat products [11]. The difference in taste, texture, appearance, and odor of plant protein based foods comparing to meat and meat based products might hinder the enhancement of consumption of plant protein based products. So, the sensory properties of the meat analogues must be optimized for the wide acceptance among consumers. However, few products (e.g., veggie burgers, black bean burgers) that do not imitate meat are also included under the definition "meat analogues", as they are also produced with the intention to replace meat, such as beef, chicken, or fish patty [12]. Meat analogues are commonly produced from plant based protein ingredients such as soya, wheat and pea, but also few products are made from other proteins sources, e.g. milk, egg, and fungal substrates. Early meat analogue products, such as tofu or seitan are produced with empirical technologies. Since 1960s, soya and wheat protein ingredients were used to develop meat analogues using low moisture (< 35%) extrusion. Since the low moisture extruded traditional meat analogues lacked the texture of meat, HME has been introduced in the early 1980s. HME showed potential to impart the fibrous texture and mouth feeling in meat analogues similar to meat [13]. Currently, extrusion is the most commonly used commercial technique for the texturization of plant proteins. Other techniques that can be used for protein texturization are wet spinning [14], electro spinning [15] [16], freeze structuring and shear cell technology [17] [18] [19]. However, these technologies are not yet well researched and require some more time to be functional on commercial level.

1.3. High moisture extrusion (HME)

The extrusion process is defined as a continuous thermomechanical process by which the protein /starch based food materials can be moistened, molten, plasticized and expelled out of the die through the combination of pressure, heat, and mechanical shear [20]



Protein texturization using high moisture extrusion [21]

According to Noguchi [22] extrusion cooking is called HME cooking, if the processed mass shows a moisture content of above 50 %, whereas Cheftel et al [23] und Akdogan [24] defined the HME process with a minimum moisture level of 40 %. Unlike in low moisture extrusion, in HME the shear produced in the extruder is low due to the lower viscosity produced by the high moisture content. Hence, the thermal energy must be efficiently incorporated in the extruder to increase the feed temperature. For HME, extruders are normally configured with a high length/diameter ratio between 25 and 40 [25] . Furthermore, twin screw extruders are usually used for HME as they provide better conveying mechanisms and enable the better process controlling than single screw extruders [22] [24]. However, as part of this dissertation, the feasibility of a planetary roller extruder was evaluated for the soya protein meat analogues. This type of extruder produces less shear and is normally used for mixing purposes and for food products which require less shear, for example ice cream.

For the texturization of proteins using HME, the cooking zone of the extruder is heated to a temperature between 130 to 170 °C. At this temperature protein based material is plasticized under higher shear and pressure. A long cooling die attached at the end of extruder barrel allows the plasticized mass to cool down to a temperature below 100 °C. The mass is pushed through the die in a laminar flow where the stream alignment and fibrous structure formation take place [21].

The biopolymers used in extrusion are normally in the state of amorphous or partially crystalline solid structures, which depends on the microstructure and composition of the biopolymers. During extrusion heating, the polymers are converted from the solid to the liquid state due to the glass transition. In case of partially crystalline polymers, a liquid condition occurs through melting. The converted materials are turned again to solid or rubbery state in the cooling die, which is different from the original solid state of the biopolymers [25]. It was also reported that during extrusion the protein molecules are unfolded due to the hydrolysis of peptide bonds and realigned through the modification of amino acid chains. Finally, the molecules are reconnected in the cooling die and forms fibrous structures through covalent iso-peptide crosslinking [24] [26].

Many studies focused on explaining the correlation between protein reactions in the extruder and textural properties of the extrudates using protein solubility tests and showed no indications for the intermolecular peptide bonding [26]. It was reported that the disulfide bonds could play more important role than non-covalent interactions in stabilizing the structure of the extrudates [26] [27], whereas Chen et al [28] found that non-covalent bonds contributed more than covalent ones. So, the disagreement still exists on which linkages are more important for the extrudate structure formation.

1.4. Protein ingredients for the high moisture extrusion

In the current research, protein ingredients, such as soya, lupin and *Spirulina platensis* were examined.

Soya:

Soya is the most researched protein for meat analogues using the HME process [29]. Defatted soya meal, soya protein concentrate and soya protein isolate were suggested for HME. Full-fat soya flour or any other whole plant based flour with high fat content is not preferable for HME, as the high fat content produces less shear in the extruder and affects the texturization negatively. Moreover, other problems such as oil separation and lower oxidative stability can occur due to the higher fat content.

The formulation of meat analogues usually contains a plant protein concentrate or isolate, insoluble fiber, starch and other ingredients for taste and texture. Early in the 1980s, HME was mainly performed with defatted soya flour [30] [31] [32]. In the later years, soya protein isolate was mixed with other ingredients such as wheat gluten and wheat starch and used for HME [33- 40] .

Lupin:

Lupin is a plant protein source comparable to soya bean, and it is already used in food products in Europe. There has been a slight increase in lupin cultivation area and production rate in Europe over the past years. However, compared to soya or pea ingredients, the application of lupin exists only to a smaller extent despite having numerous beneficial properties. In bakery and gluten free products, lupin is applied in very low concentration (<5 %). However, development of new products containing lupin ingredients is increasing these days. For growing sectors, such as vegetarians, vegans, and people with intolerance or allergy to gluten, soya, milk, or egg, there is huge potential for lupin-based products [41]. The extrusion of lupin ingredients has not been researched until to date, and the potential meat analogues with lupin protein can be regarded as regional in Europe, as lupin can be cultivated locally.

***Spirulina*:**

Microalgae are considered as arising novel sustainable alternative protein sources. It is predicted that by the middle of this century, 18 % of the whole protein sources might be covered by algae in the diverse markets [42]. Microalgae are also regarded as an alternative for other plant proteins due to allergenicity issues [43]. Among different species of microalgae, *Spirulina* and *Chlorella* are the most used in the food sector due to their high protein content and excellent nutritional value [44].

The potential of microalgae has been already well realized in Europe. A *Spirulina* based burger was launched in Dutch and Belgian markets already [45]. The very first study by Grahl et al reported the sensory aspects of meat analogues produced from soya and *Spirulina* mixtures. The study showed that in the soya protein based meat analogues produced by the HME process, 50 % of the soya protein can be replaced with *Spirulina platensis* biomass [46]. Apart from the potential use in meat analogue products, recently the incorporation of microalgae in meat products has also been investigated [41] [45]. It was found that the addition of *Spirulina* increased the total, essential and non-essential amino acid content in pork sausages [43].

Other protein ingredients:

Pea protein also gained attention as an alternative protein in recent years for the HME processing, as it shows high nutritional and low allergenic characteristics [27] [48]. Apart from soya and pea, other ingredients, such as wheat gluten [49] [50] [51], peanut protein [52] [53] [54] has also been researched with HME. Other legumes and proteins from oilseed sources could also be in the ingredients list in the future for the meat analogues production with HME [55].

1.5. Control of texturization

The texturization quality of meat analogues can be assessed by analyses, such as cutting force measurements [33] [38] [40], microscopical characterization [22] [34] [56] and sensory evaluation.

The measurement of the cutting force is performed in longitudinal and transverse directions with respect to the flow direction in the cooling die in order to determine the degree of texturization/fiber formation. Other methods for the determination of fiber formation include fluorescence polarization spectroscopy [57] and a real-time scanning system based on photon migration [35]. Nevertheless, sensory evaluation is considered to be the best analysis to evaluate the meat analogues, as it provides the data for overall textural quality characteristics. The fibrous structure formation/ texturization is a key quality characteristic for the meat analogues which provides the mouth feeling and texture similar to meat. Texturization can be controlled by several factors, such as by system parameters inside the extruder and cooling die and the composition of the raw materials [21].

Moisture content appears to be an important parameter affecting the overall texture quality of the product. The HME of soya protein with lower moisture content showed higher die pressure, and the products resulted in harder texture, lower total protein solubility, and the products were tougher, chewier, and more cohesive [33] [34].

The texture of the meat analogues can also be controlled by the extrusion cooking temperature [22] [38] [48] [58]. The texturization temperature and moisture vary for each protein type and raw material composition. The texturization begins to occur for the pea protein isolate at a temperature between 100 and 120 °C with an optimum of around 140 °C [48]. The experiments conducted with differential scanning calorimetry by Noguchi [22] showed that for the mixture of soya flour with 60 % moisture, the temperature of 130 °C was considered the lowest for the texturization. However, according to the study of Lin et al [33], the HME of soya protein isolate at 130 °C showed fibrous structure, which could be due to the different raw material composition (e.g. protein content). Besides the raw material composition, the optimum extrusion conditions also depend upon the extruder screw configuration. The cutting force of the extrudates can be increased with increased shear by using aggressive screw configurations [40].

1.6. Texturizing aids

Effect of additives on the properties of meat analogues with HME is still an unexplored area. The sulfur containing additives can be used for the HME as studied for low moisture extrusion of soya [59]. The effect of additives, such as metasodium bisulphite, cysteine and phosphate was investigated by Roberts [60] and it was shown that only phosphate improved the texturization of wheat gluten under low moisture extrusion.

The application of polysaccharides in the protein based extrusion improved the texturization, which perhaps occurs through a phase separation phenomenon between protein and carbohydrate during the stream alignment in the die. Transversal protein aggregation is hindered through the phase separation which results in the maintenance of individual protein fibers [23]. Contrary to this hypothesis, Noguchi [22] hypothesized that protein-protein interactions could be enhanced in longitudinal direction by water binding polysaccharides. It was also reported that a mixture of two noncompatible proteins or polysaccharides enable phase separation and fiber formation through thermodynamic noncompatability caused by electrostatic repulsions [61]. Several conditions, such as pH, presence of ions, and concentration of each phase affect the phase separation. Respective viscosities of the two phases, the interfacial tension, and the rate of gelation of one or both phases also control and influence the fiber formation [23]. Boison et al [62] tested sodium alginate and methyl cellulose at the concentrations of 0.5 % and 1 % levels for the low moisture extrusion of defatted soya flour and reported that the sodium alginate increased the texture attribute “maximum peak force”, whereas the methylcellulose showed the opposite results and weakened structure formation. Berrington et al [63] tested several hydrocolloids with low moisture extrusion of defatted soya grits. Hydrocolloids, such as guar gum, locust bean gum, sodium carboxymethyl cellulose, pectin and carrageenan had little effect on the extruder response on 1 % level, whereas sodium alginates, hydroxypropyl and hydroxyethyl celluloses showed significant reduction in extruder torque and product temperature. When defatted soya grit was extruded with 1 % sodium alginate at 180 °C, a decreased expansion ratio of the extrudate was observed. It was further reported that the molecular mass of the polysaccharides also influenced their reaction with protein [63]. However there have been no studies performed until to date regarding the effect of polysaccharides on HME. Hence, a good understanding of protein and polysaccharides interaction during extrusion is still required to control and improve the fiber formation.

1.7. Effect of extrusion on nutritional properties

The antioxidant properties and protein digestibility of the meat analogues were also examined as part of this research. Generally, extrusion is considered as a high temperature short time process and advantageous for the protection of the food components, and the undesirable effects on proteins, amino acids, starch and vitamins can be restricted [64]. Studies on starch based extruded snacks revealed that the extrusion can also positively affect the content of bioactives [65] [66] or their bioavailability [67].

Several factors such as raw material characteristics, mixing and conditioning of raw material, barrel temperature, pressure, screw speed, moisture content, flow rate, energy input, residence type, screw configuration etc can influence the final composition of the extrudates [68]. Even though the extrusion process is considered as a short time process, the mechanical and thermal stresses caused during the short time process can have detrimental effects on the bioactive compounds [69].

The extrusion process was also reported to have a positive effect on the protein digestibility. Low moisture extruded soya and rape seed meals showed higher protein digestibility than untreated meals [70]. There have been no studies conducted on the protein digestibility of HME based protein products. However, the study by MacDonald et al [36] reported that the protein digestibility of HME based soya protein extrudates seemed to be higher, as the products showed higher growth rate in the tested mice.

2. Objectives of the work

The main objective of this work was to develop and evaluate meat analogues from alternative protein sources using HME processing. In the first part of the work, mixture of lupin concentrate and isolate was extruded and the focus was here to investigate the effect of extrusion conditions on system parameters and product properties (physical, textural and IVPD).

As a follow up to the first part of the research, *Spirulina platensis* biomass was incorporated with lupin mixtures, and the physico-chemical properties were evaluated. The additional focus was here placed on the evaluation of antioxidant properties of *Spirulina* enriched meat analogues other than textural and physical characteristics.

Further research work aimed to investigate the potential of using additives for the texture improvement of soya meat analogues. Here, the focus was also to use a planetary roller extruder (PRE) than traditional twin screw extruder. The objective of this research part was to examine the feasibility of a PRE extruder and also the effect of application of ICGN on physical, textural and sensory properties of the meat analogues.

3. Publication: High moisture extrusion of lupin protein: influence of extrusion parameters on extruder responses and product properties

Preface

Investigating new alternative protein sources is necessary to broaden the variety of meat analogues and to create value addition for those protein sources. The aim of this work was to investigate the effect of extrusion parameters on extruder responses and the lupin meat analogue properties. Though other earlier studies reported such effects on other proteins, such as soya and pea, a generic study is essential to understand how the lupin ingredients function in the extruder and to check the feasibility of lupin protein for the HME. Preliminary studies found the correct ratio of lupin concentrate and isolate, which was used in the study. Furthermore, the lack of knowledge on the protein digestibility of meat analogues also prompted this study first time to investigate the IVPD. Other than checking the feasibility of lupin proteins for HME, the textural and physical properties of the extrudates must be studied in relation to the extrusion conditions. As described in the references section, the HME studies of soya or peas showed that the extrusion conditions (temperature/water feed content/screw speed) have direct impact on the extruder responses (die pressure, product temperature, torque and specific mechanical energy) which in turn influence the extrudate properties. Knowledge on such effects regarding extruder responses and product properties must also be generated for a lupin meat analogue. Thus, the objective of the study was to investigate the effect of extrusion conditions on extruder responses and extrudate properties such as textural, physical, microstructural aspects and IVPD.

In this study, Knut Franke was involved in the setup of the experimental plan and contributed the statistical analysis. Stefan Töpfl, Ralf G. Berger and Volker Heinz contributed project ideas and were involved in the manuscript preparation process.

Research Article

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High moisture extrusion of lupin protein: influence of extrusion parameters on extruder responses and product properties

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Abstract

BACKGROUND: High moisture extrusion (HME) of lupin protein concentrate and isolate (50:50) mixture was performed by varying the extrusion parameters, such as barrel temperature (138–180 °C), water feed (40–68%) and screw speed (400–1800 rpm). The effect of extrusion parameters on extruder responses [die pressure, product temperature, torque and specific mechanical energy (SME)] and product properties [colour, cutting force, cooking yield, microstructure and *in vitro* protein digestibility (IVPD)] was evaluated.

RESULTS: The multiple regression analysis of the results revealed that the water feed had a significant negative linear effect on the extruder responses considered, as well as on colour difference and cutting force of extrudates. Screw speed had a positive linear effect on product temperature, SME and cooking yield. Barrel temperature affected extruder responses and product properties to a lesser extent. Scanning electron microscopy showed that denser microstructure and higher number of fibre layers were created by increasing temperature and screw speed along with decreasing water feed. The results of IVPD of selected extrudates showed that the increase in barrel temperature decreased the IVPD, whereas the increase in water feed resulted in higher IVPD. The screw speed had no significant effect on IVPD.

CONCLUSION: The study demonstrates that the use of lupin protein is feasible to produce meat analogues with HME which could enhance the possibilities to meet the growing protein demands for human consumption.

Keywords: high moisture extrusion; lupin protein; extruder responses; meat analogue properties; protein demand

INTRODUCTION

The global protein demand keeps on increasing not only due to the rising population, but also because of economic development, increased urbanization and increasing awareness of protein in a healthy diet. Moreover, importance of plant proteins is growing over the animal proteins due to their favourable environmental and sustainability aspects.[1,2] Among plant proteins, pulses are considered as one of the staple sources of proteins in some parts of the world (e.g. some Asian and South American countries). In recent years lupin has been one of the pulses gaining attention worldwide. Three varieties of lupin, such as white lupin (*Lupinus albus*), yellow lupin (*Lupinus luteus*) and narrow-leafed blue lupin (*Lupinus angustifolius*) are native to Europe[3] and the latter one was used in this study. Sweet lines are commercially available for all three of these varieties. The lupin protein comprises 90% of globulins and 10% of albumins, whereas the other protein factions, such as prolamins and glutelins, are present in very small amounts or absent.[4] Lupin protein can be incorporated in food products such as cakes, pancakes, biscuits, pasta and bread to replace egg or to improve the protein content.[5] Lupin based milk and yoghurt alternatives have also been in the focus recently.[6,7]

In this study, an attempt was made to develop texturized lupin protein meat analogues using high moisture extrusion (HME) processing. Texturization of plant proteins such as soya, pea and wheat gluten with HME have been investigated extensively in the past.[8–12] However, the application of other plant proteins in HME is necessary to broaden the variety of meat analogues and their resources. In the HME process, high temperatures between 130 and 170 °C are applied in the extruder barrel, and the water feed is usually set between 40% and 70%. According to Onwulata *et al.*,[13] the protein cross-linking and the formation of a solidified three-dimensional network occur in three steps. In the first step, during the HME cooking, proteins undergo swelling, dissolving, and unfolding in the extruder cooking zone. In the second step, intermolecular covalent bonds are formed between the accessible reactive groups of the proteins. The third step occurs in the cooling die, where the cooling process along with the shear and elongation forces is responsible for the stabilization of aligned protein molecules or small aggregates. Moreover, the non-covalent

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Table 1. Design of experiment for the trials

Run number	Coded levels x_1	x_2	x_3	Actual levels Barrel temperature (°C)	Water feed (%)	Screw speed (min^{-1})
1	−1	1	1	145	62	1600
2	−1	1	−1	145	62	800
3	0	0	0	155	55	1200
4	$+\alpha$	0	0	180	55	1200
5	0	$+\alpha$	0	155	68	1200
6	1	1	1	165	62	1600
7	−1	−1	1	145	47	1600
8	1	1	−1	165	62	800
9	0	0	$+\alpha$	155	55	1800
10	1	−1	1	165	47	1600
11	0	0	0	155	55	1200
12	−1	−1	−1	145	47	800
13	0	0	0	155	55	1200
14	1	−1	−1	165	47	800
15	0	$-\alpha$	1	155	40	1200
16	$-\alpha$	0	0	135	55	1200
17	0	0	$-\alpha$	155	55	400

intermolecular electrostatic and hydrophobic interactions occurring during cooling also stabilize the protein network.[13]

To our knowledge, no texturization studies of lupin protein by HME exist. In the current study, a mixture of lupin protein concentrate and isolate was processed using HME, and the effects of extrusion parameters on extruder responses and product properties were investigated.

MATERIALS AND METHODS

Raw materials

Lupin protein isolate was procured from Wellness & Healthcare Service GmbH, Neuenbürg, Germany. Lupin protein concentrate was purchased from Frank Food Products, Twello, the Netherlands.

Proximate composition

The proximate composition (moisture, protein, fat and ash content) of lupin protein concentrate and isolate was determined according to German official methods (§ 64 LFGB). The carbohydrate content was calculated by the difference method.

High moisture extrusion (HME)

The HME of lupin protein concentrate and isolate mixture (50:50) was carried out using Coperion ZSK 27 Mv PLUS twin screw extruder (Coperion GmbH, Stuttgart, Germany). The length of the extruder barrel was 1000 mm and the diameter of screws inside and outside was 26.7 mm and 15.2 mm, respectively. A cooling die was attached at the end of the extruder barrel. Internal dimensions of the cooling die were $50 \times 15 \times 800\ mm^3$ [width (W) × height (H) × length (L)]. The extruder barrel has nine heating zones. Only barrel temperatures of zones 5, 6 and 7 were modified for the trials. Temperatures of zones 1, 2, 3, 4, 8 and 9 were kept constant and set to 40, 60, 90, 120, 140 and 120 °C, respectively. Water temperature entering the jacket of the cooling die was set to 30 °C using a cooling bath and was constant for all the trials. The dry raw material mixture was fed into the extruder at the feed rate of 8 kg h^{-1}. Water feed rate and screw speeds were adjusted for the trials according to the design of experiments (Table 1). Once the extrusion process reached a steady state, the extruder responses torque, die pressure and product temperature were recorded. Product temperature and die pressure were measured at extruder exit before the product entered the cooling die. The samples were collected from the end of cooling die, vacuum packed and stored at −20 °C until analyses. Before analysis, samples were thawed in a refrigerator overnight and placed at room temperature for 3 h except for *in vitro* protein digestibility (IVPD), where freeze dried samples were used.

Motor torque and specific mechanical energy (SME)

The percentage of torque was recorded by the extruder control unit and was subtracted from the torque of a no-load run at a given screw speed (idle torque). This corrected percentage torque was transferred into real corrected torque using the maximum achievable motor torque (200 Nm). This real corrected torque was later only named as torque and used to calculate the specific mechanical energy (SME) using the formula:[14]

$$SME = \frac{2 \cdot \pi \cdot n \cdot D}{MFR} \qquad (1)$$

where n indicates screw speed, D is torque, and MFR is the product flow rate.

Product properties

Colour

Colour measurements were performed for the thawed extrudates and untreated raw material mixtures using a spectrophotometer (CM-600, Konica Minolta Sensing Inc., Japan). The instrument records the L^* (lightness), a^* (green-red) and b^* (blue-yellow) values. Ten measurements were taken at different surface points for each of the samples. Total colour difference (ΔE) of the extrudates was calculated using the equation:[15]

$$\Delta E = \sqrt{\left(L - L_0\right)^2 + \left(a - a_0\right)^2 + \left(b - b_0\right)^2} \qquad (2)$$

The subscript zero indicates the colour parameters of the raw material mixtures.

Cooking yield

The thawed samples were cut into cubes with a size of 20 × 20 × 15 mm^3 ($L \times W \times H$) and heated for 20 min in water at 80 °C. After cooking, the samples were removed from the water and placed on a sieve at room temperature for 10 min to remove the surplus surface water completely. The mass before and after heating was measured and cooking yield was calculated using Eqn (3):

$$Cooking\ \ yield = \frac{m_{CS}}{m_{RS}} \cdot 100\% \qquad (3)$$

where m_{CS} is the mass of the sample after cooking, whereas m_{RS} means the mass of the sample before cooking. The analysis was carried out in triplicate.

Cutting force

The cutting force was measured using a Texture Analyser (Stable Micro Systems TA XT 2, Godalming, UK). The system was calibrated with a 5 kg cell. The thawed samples with size of 20 × 20 × 15 mm^3 ($L \times W \times H$) were taken and cut using a razor blade with a pre-test speed of 1.0 mm s^{-1}, a test speed of 2.0 mm s^{-1} and a post-test speed of 10 mm s^{-1}. The samples were cut in the transverse direction of the die exit, and the maximum force was used as the measure for cutting force. Five measurements were taken for each sample.

Microstructure

The microstructure of the meat analogues was analysed using scanning electron microscopy (SEM). The samples were cut into the size of 3 mm width and 10 mm length, frozen in liquid nitrogen slush and broken. Free water from the fracture surface of the sample was removed at −8 °C and a pressure of 10^{-4} mbar for 4 h using an Emitech K1250 Cryo-SEM system. Then, the sample surfaces were sputter coated with gold and images were obtained using a Cryo-SEM (JEOL JSM- 6460 LV, Tokyo, Japan) at −180 °C.

In vitro *protein digestibility (IVPD)*

IVPD of selected freeze-dried lupin extrudates was determined using a modified pH-drop procedure as described by Tinus *et al.*[16] Briefly, the sample weight equivalent to 62.5 mg protein was added to 10 mL of distilled water and incubated at 37 °C for 1 h in a shaking incubator. Then, the pH of the suspension was adjusted to 8.0 approximately using 0.1 mol L^{-1} sodium hydroxide (NaOH) and/or 0.1 mol L^{-1} hydrochloric acid (HCl). A multi-enzyme solution consisting of 16 mg trypsin, 31 mg chymotrypsin and 13 mg protease was prepared. Enzyme solution was kept at 37 °C while the pH was adjusted to 8.0 as described earlier. Then, 1 mL of multi-enzyme solution was added to 10 mL of the sample suspension. The pH value of this blend was measured immediately after addition of the multi-enzyme solution and after 10 min. The analysis was conducted in triplicate. IVPD was calculated using the following equation: IVPD = 65.66 + 18.10 × ΔpH_{10min}, where the ΔpH_{10min} is the change in pH value after 10 min from the initial pH value.

Statistical analysis

The design of the experiment was created based on the 2^3 factorial design (Table 1). Correlations and regression coefficients were calculated using a self-written statistic program DataStar 3.4. IVPD data were analysed by one-way analysis of variance (ANOVA) to determine the statistical significance ($P < 0.05$).

Multiple linear regression was used to analyse the effect of extrusion parameters on extruder responses and product properties. For this purpose, a second-order polynomial (Eqn (5)) was applied to calculate the effects of extrusion temperature (x_1), water feed (x_2) and screw speed (x_3).

$$Y = b_0 + b_1^* x_1 + b_2^* x_2 + b_3^* x_3 + b_4^* x_1^* x_1 + b_5^* x_2^* x_2 + b_6^* x_3^* x_3 + b_7^* x_1^* x_2 + b_8^* x_1^* x_3 + b_9^* x_2^* x_3 \qquad (5)$$

During calculation, non-significant coefficients in Eqn (5) were removed automatically ($P > 0.90$). Thus, final regression equations contain only statistically significant coefficients.

Table 2. Proximate composition of raw materials

Composition (g kg^{-1})	Lupin protein isolate	Lupin protein concentrate
Moisture	55.1	68.7
Protein	778	511
Fat	37.3	99.4
Ash	49.8	31.9
Carbohydrate	79.8	289

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the raw materials is listed in Table 2.

Influence of extrusion parameters on extruder responses

Table 3 gives a complete overview of the settings and results of all extrusion experiments. As described in the statistical analysis section, these data were used in a first step to calculate correlations between extrusion parameters (columns 1–3), extruder responses (columns 4–7) and product properties (columns 8–13). All correlation coefficients are presented in Table 4.

The calculated influence of extrusion parameters on extruder responses and product properties based on Eqn (5) was used to establish the three-dimensional diagrams, where two of the three extrusion parameters are assigned to the *x*- and *y*-axis, whereas the third one was set to constant as shown in Figs 1–7.

Influence on die pressure

The results of the influence on die pressure are shown in Fig. 1. Die pressure values ranged from 0.6 to 3.4 MPa. The extruder parameters barrel temperature and water feed had a negative correlation with the pressure. The increase in barrel temperature and water feed tended to reduce the viscosity of the material inside the extruder, thereby decreasing the pressure at the die.[8] The influence of water feed on die pressure was more pronounced than that of the barrel temperature (Fig. 1(A)). It was observed that increasing screw speed led to a slight increase in die pressure. It can be assumed that increasing screw speed could intensify the protein texturization by increased cross-linking of the protein molecules in the extruder cooking zone, resulting in firmer consistency as well as increased die pressure. However, this effect could be limited by a shorter residence time of the material in the extruder due

Table 3. Effect of extrusion parameters on extruder responses and product properties

Independent extrusion parameters			Extruder responses				Product properties					
Barrel temperature (°C)	Water feed (%)	Screw speed (min^{-1})	Die pressure (MPa)	Product temperature (°C)	Torque (N m)	SME (kJ kg^{-1})	L^*	a^*	b^*	ΔE	Cutting force (N)	Cooking yield (%)
145	62	1600	1.0	120.1	4.07	307	55.4 ± 0.95	7.36 ± 0.31	24.5 ± 3.99	32.1 ± 0.89	6.90 ± 0.19	114.5 ± 0.65
145	62	800	0.8	114.0	3.87	146	54.8 ± 0.70	8.66 ± 0.39	24.0 ± 2.17	32.8 ± 0.82	7.24 ± 0.24	109.8 ± 0.19
155	55	1200	1.3	121.2	7.13	403	49.4 ± 1.94	8.01 ± 0.39	23.0 ± 1.46	37.5 ± 1.78	11.94 ± 0.61	118.5 ± 0.70
180	55	1200	1.1	121.4	5.12	289	53.3 ± 1.05	9.07 ± 0.29	24.8 ± 2.09	34.6 ± 1.08	13.23 ± 1.69	114.5 ± 0.93
155	68	1200	0.6	114.7	0.94	53.4	55.8 ± 1.18	7.75 ± 0.32	24.0 ± 2.87	32.0 ± 1.26	3.77 ± 0.19	119.7 ± 0.81
165	62	1600	0.8	121.7	4.14	312	56.8 ± 1.29	7.67 ± 0.47	23.5 ± 3.43	30.5 ± 1.19	5.84 ± 0.38	114.7 ± 0.42
165	62	1600	0.8	121.7	4.14	312	56.8 ± 1.29	7.67 ± 0.47	23.5 ± 3.43	30.5 ± 1.19	5.84 ± 0.38	114.7 ± 0.42
145	47	1600	2.5	131.9	11.00	829	51.8 ± 0.91	7.67 ± 0.39	19.3 ± 2.36	35.8 ± 0.90	19.97 ± 3.60	124.3 ± 0.44
165	62	800	1.0	115.5	4.47	168	55.1 ± 0.98	8.57 ± 0.29	26.0 ± 1.32	32.5 ± 0.94	6.71 ± 0.30	112.4 ± 0.65
155	55	1800	1.6	127.2	8.15	691	54.5 ± 1.27	8.49 ± 0.38	18.9 ± 2.34	33.2 ± 1.29	15.70 ± 0.58	116.1 ± 0.51
165	47	1600	2.6	130.2	13.34	1006	51.7 ± 1.29	8.49 ± 0.39	21.6 ± 1.80	35.8 ± 1.36	18.37 ± 0.70	121.8 ± 0.34
155	55	1200	1.4	121.7	6.82	385	53.5 ± 1.28	9.17 ± 0.33	22.5 ± 2.77	34.2 ± 1.20	11.51 ± 0.37	117.7 ± 0.72
145	47	800	2.4	121.7	11.45	431	49.3 ± 0.66	9.76 ± 0.46	18.9 ± 1.73	38.6 ± 0.70	16.55 ± 2.17	118.0 ± 0.82
155	55	1200	1.3	121.7	6.64	375	54.9 ± 0.61	8.60 ± 0.35	25.6 ± 2.93	32.9 ± 0.73	11.45 ± 0.61	119.0 ± 0.71
165	47	800	1.7	122.3	10.18	384	50.3 ± 1.08	9.37 ± 0.43	20.6 ± 2.10	37.3 ± 1.13	19.49 ± 0.43	113.7 ± 0.78
155	40	1200	3.4	130.2	14.00	792	50.8 ± 2.44	8.11 ± 0.82	22.7 ± 2.23	36.7 ± 2.48	23.60 ± 2.03	119.9 ± 0.67
135	55	1200	1.7	122.3	7.56	427	52.6 ± 1.73	8.38 ± 0.48	20.9 ± 2.59	34.9 ± 1.73	9.39 ± 1.76	132.2 ± 1.06
155	55	400	0.8	112.0	6.61	125	53.5 ± 1.15	9.13 ± 0.41	22.9 ± 2.43	34.2 ± 1.25	9.93 ± 0.51	104.7 ± 0.14

Note: SME, specific mechanical energy; colour measurements: L^*, lightness value; a^*, green-red value; b^*, blue-yellow value; ΔE, total colour difference.

Table 4. Correlation coefficients

	Barrel temperature	Water feed	Screw speed	Die pressure	Product temperature	Torque	SME	L^*	a^*	b^*	ΔE	Cutting force	Cooking yield
Barrel temperature	–	0.00	0.00	−0.15	0.00	−0.07	−0.04	0.102	0.203	0.361	−0.1	0.08	−0.39
Water feed		–	0.00	−0.91***	−0.72***	−0.97***	−0.76***	0.797 ***	−0.34	0.577 **	−0.812 ***	−0.96 ***	−0.29
Screw speed			–	0.22	0.67***	0.10	0.58**	0.217	−0.637 ***	−0.198	−0.247	0.13	0.49**
Die pressure				–	0.83***	0.94***	0.86***	−0.684***	0.087	−0.571 **	0.693***	0.89***	0.50**
Product temperature					–	0.77***	0.95***	−0.426*	−0.197	−0.548 **	0.417*	0.79***	0.60**
Torque						–	0.85***	−0.764***	0.267	−0.633***	0.769***	0.84***	0.35
SME							–	−0.47*	−0.126	−0.574**	0.458*	0.90***	0.52**
L^*								–	−0.402	0.561 **	−0.995***	−0.74***	−0.28
a^*									–	−0.232	0.459*	0.27	−0.29
b^*										–	−0.576**	−0.60**	−0.37
ΔE											–	0.76***	0.27
Cutting force												–	0.26
Cooking yield													–

Note: SME, specific mechanical energy.
Colour measurements: L^*, lightness value; a^*, green-red value; b^*, blue-yellow value; ΔE, total colour difference.
Significance levels: ***$P > 0.99$; **$P > 0.95$; *$P > 0.90$.

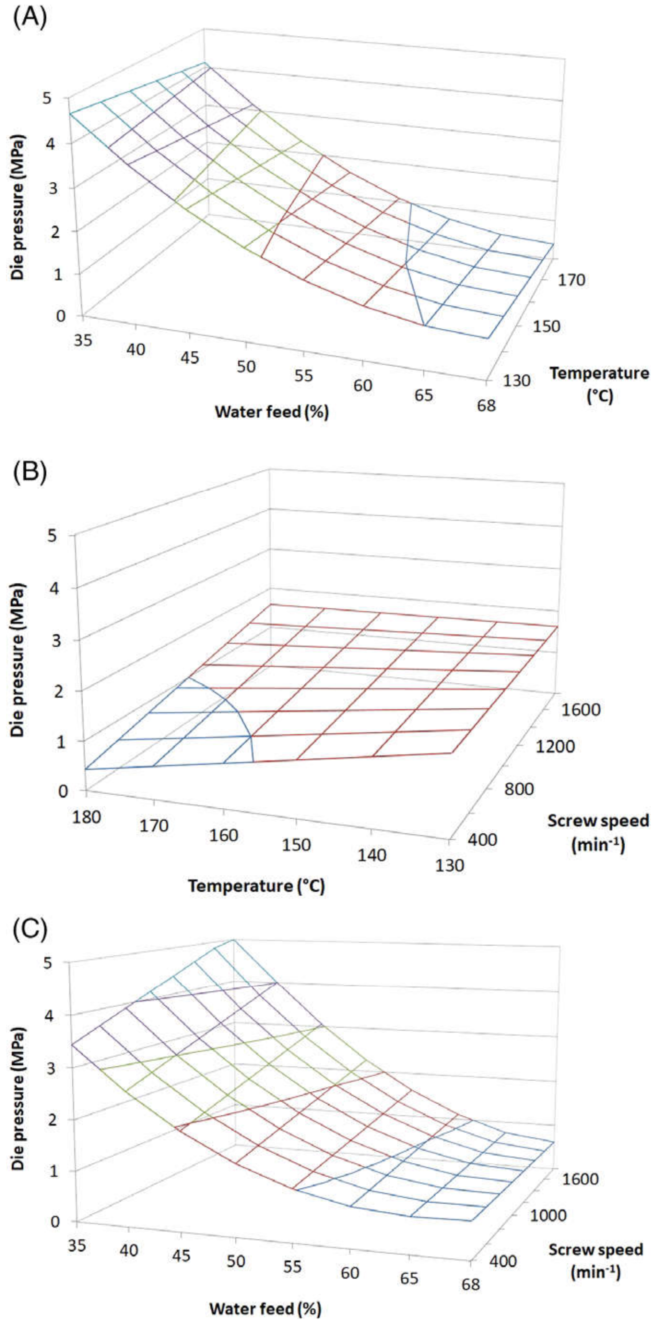

Figure 1. Calculated influence of two variable and one constant extrusion parameters on die pressure (settings for constant parameters: (A) screw speed = 1200 min^{-1}; (B) water feed = 55%; (C) temperature = 155 °C).

to the increasing screw speed which may restrict the time for the cross-linking reaction.[17] But the increased cross-linking and protein denaturation effect overruled the effect of shorter residence time with the increased screw speed.

The effect of screw speed on die pressure was noticeable at higher barrel temperatures rather than at low barrel temperatures (Fig. 1(B)). It is assumed that the higher barrel temperature could lead to more protein denaturation and cross-linking along with the increase in screw speed in the extruder cooking zone. Thus, the higher screw speed could have increased the die pressure to a greater extent at higher barrel temperature rather than at lower barrel temperature. This assumption is in correlation with

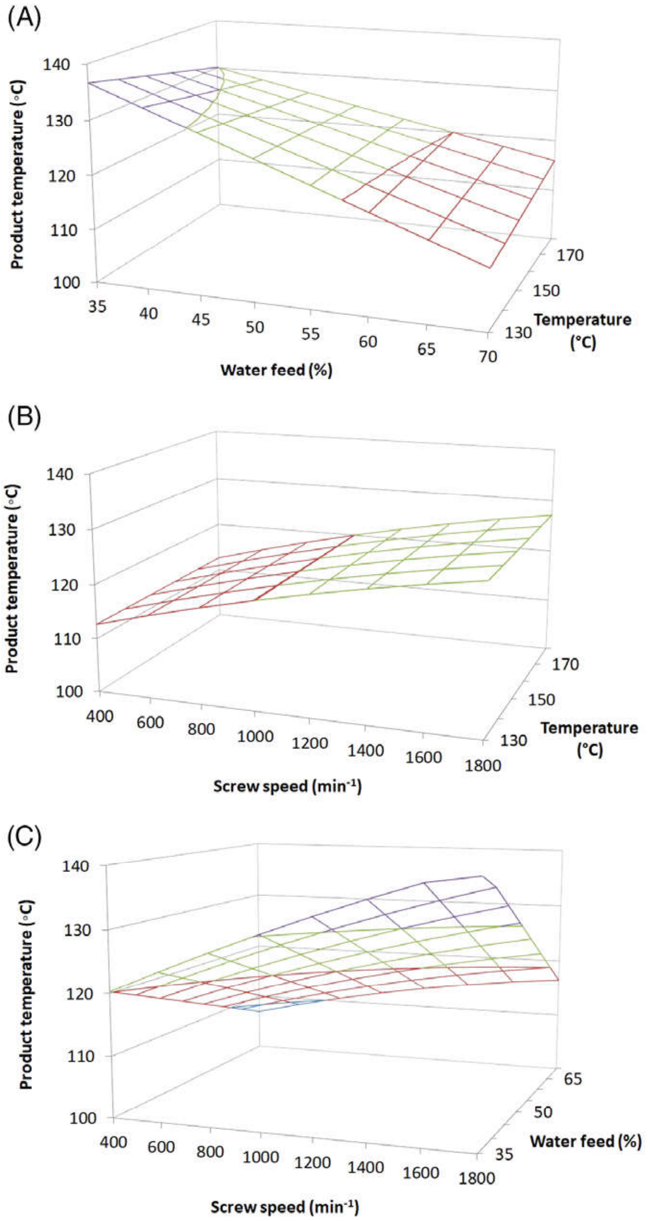

Figure 2. Calculated influence of two variable and one constant extrusion parameters on product temperature (settings for constant parameters: (A) screw speed = 1200 min^{-1}; (B) water feed = 55%; (C) temperature = 155 °C).

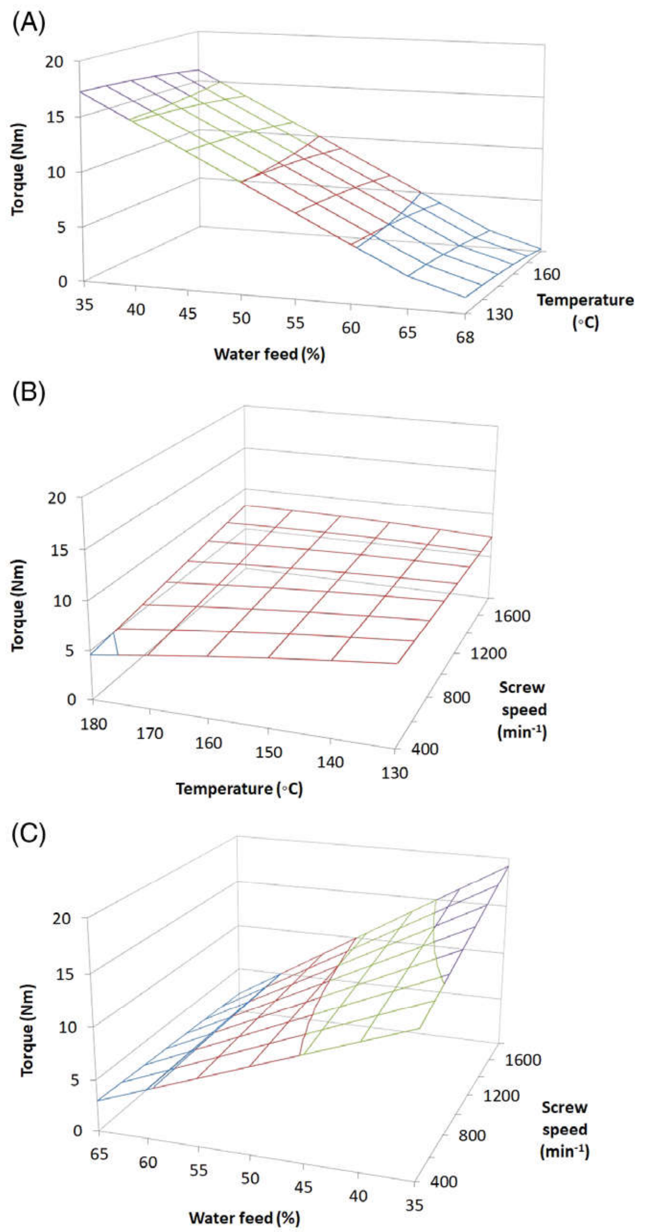

Figure 3. Calculated influence of two variable and one constant extrusion parameters on torque (settings for constant parameters: (A) screw speed = 1200 min^{-1}; (B) water feed = 55%; (C) temperature = 155 °C).

the cutting strength results. The increase in cutting strength with increase in screw speed can be attributed to a higher degree of protein texturization.

In the interaction effects of water feed and screw speed (Fig. 1(C)), the water feed had more influence on die pressure than screw speed. All in all, the highest die pressure was observed at low barrel temperature, low water feed and high screw speed levels.

Influence on product temperature

The effect of extrusion parameters on product temperature is presented in Fig. 2. Increasing the barrel temperature from 130 to 180 °C did not result in a significant increase of product temperature (Fig. 2(A)). This could be due to the fact that in our study the barrel temperature of only the middle zones were modified and the last two zones were kept constant for all the trials. As product temperature was measured at the end of the extruder, the constant barrel temperature settings in the last zones could have resulted in the very small changes in product temperature at the end of the extruder. However, at lower water feed, slightly lower product temperature was observed with increase in barrel temperature, and the opposite effect was observed in higher water feed ranges of 55% and more.

The effect of water feed on the product temperature was obvious. Increasing water feed resulted in a distinct decrease in product temperature (Fig. 2(A)). The friction between material and screw shaft was reduced due to the viscosity decrease because of increased water feed. Lower friction led to the reduction of

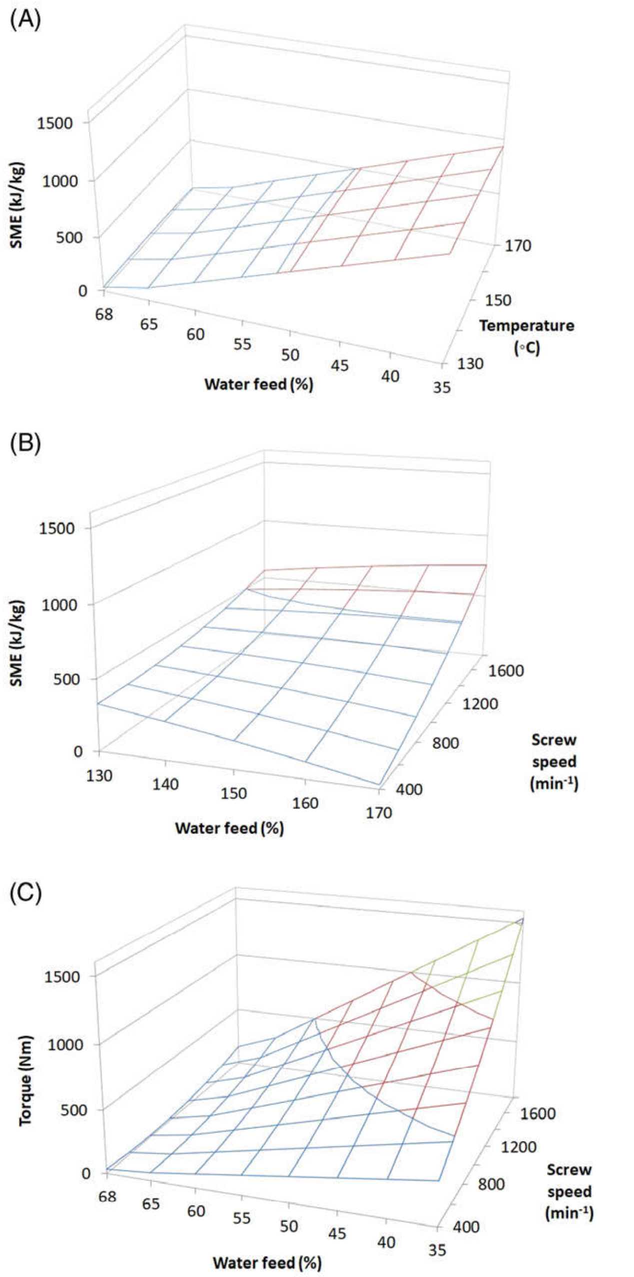

Figure 4. Calculated influence of two variable and one constant extrusion parameters on specific mechanical energy (SME) (settings for constant parameters: (A) screw speed = 1200 min $^{-1}$; (B) water feed = 55%; (C) temperature = 155 °C).

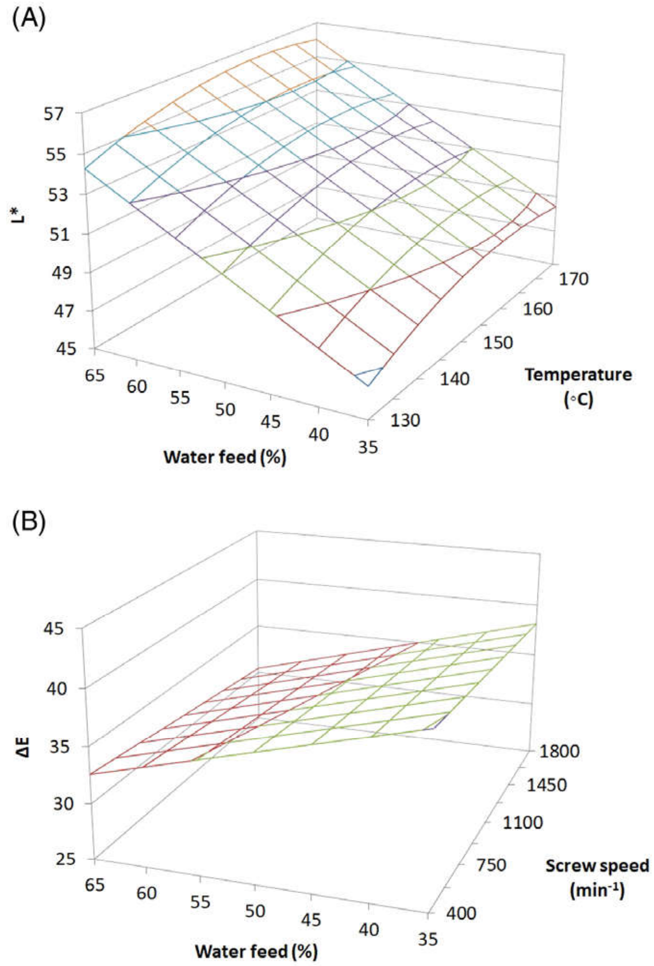

Figure 5. Calculated influence of two variable and one constant extrusion parameters on colour parameters of extrudates (settings for constant parameters: (A) screw speed = 1200 min^{-1}; (B) temperature = 155 °C).

mechanical energy dissipation ending up in reduced product temperature.[9,18]

As also observed in Fig. 2(A), product temperature was not much influenced by increasing barrel temperature also in the interaction effect of barrel temperature and screw speed (Fig. 2(B)). But increasing screw speed resulted in significant increase of product temperature. With the combined effect of water feed and screw speed, both parameters showed an effect on product temperature (Fig. 2(C)). Higher screw speed produced more mechanical energy which led to the increase in product temperature. The same behaviour was observed in earlier starch-based extrusion studies.[19,20] The highest product temperature was achieved at medium barrel temperature, low water feed and high screw speed.

Influence on torque

The results with respect to torque are shown in Fig. 3. Any changes occurring in the melt viscosity of the material during extrusion also led to the changes in pressure, torque and SME.[21] As discussed in the die pressure section, increasing barrel temperature and water feed led to a decrease in viscosity of the materials causing reduction in torque. The water feed influenced the torque more distinctly than barrel temperature in the 'temperature and water feed combination effect' (Fig. 3(A)), as was also observed with the die pressure.

In case of barrel temperature and screw speed combination (Fig. 3(B)), screw speed has no significant influence on torque. The increasing barrel temperature showed a slight decrease in the torque values only at lower screw speeds (up to 800 min^{-1}), but at higher screw speeds (from 1000 min^{-1}) the effect was very low.

With respect to water feed and screw speed combination effects (Fig. 3(C)), increasing screw speed tended to increase the torque which was in contrast to studies on starch extrusion.[16,22,23] The factors protein cross-linking and texturization due to the increased screw speed resulted in an increasing die pressure and torque. The higher the cross-linking, the higher was the amount of mechanical energy which was required to rotate the screws. In case of water feed and screw speed effects, the water feed showed the same trend as in temperature and water feed combination effect. Here,

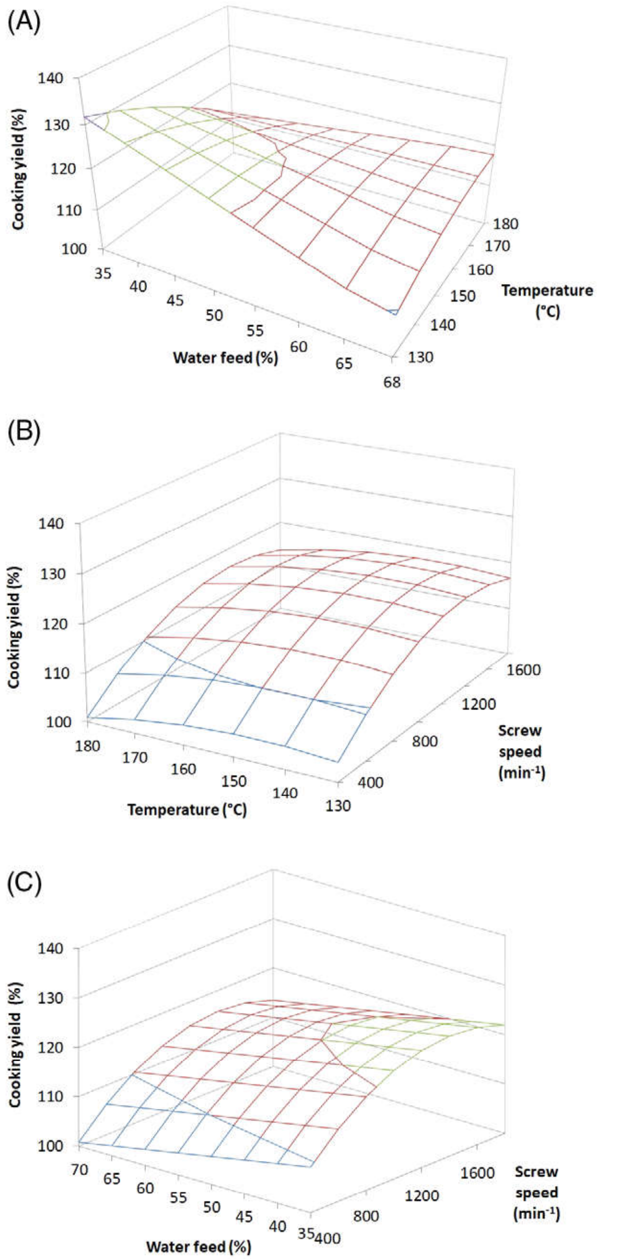

Figure 6. Calculated influence of two variable and one constant extrusion parameters on the cooking yield of extrudates (settings for constant parameters: (A) screw speed = 1200 min^{-1}; (B) water feed = 55%; (C) temperature = 155 °C).

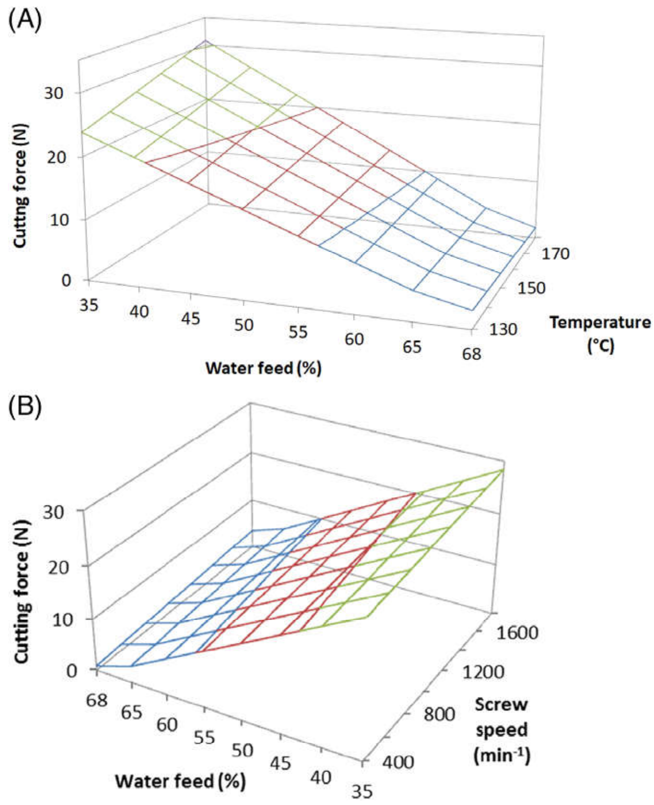

Figure 7. Calculated influence of two variable and one constant extrusion parameters on the cutting force of extrudates (settings for constant parameters: (A) screw speed = 1200 min^{-1}; (B) temperature = 155 °C).

the effect of water feed was more pronounced than screw speed (Fig. 3(C)). The highest torque was found for a combination of high barrel temperature, low water feed and high screw speed.

Influence on SME

The SME results are presented in Fig. 4. The reduced viscosity due to increased water feed and barrel temperature led to lower SME as also observed for pressure and torque (Fig. 4(A)). The effect of water feed was more obvious than that of barrel temperature in the interaction of barrel temperature and water feed (Fig. 4(A)). The decrease in SME with increasing water feed was in agreement with previous studies focused on high moisture extrusion of protein-based materials.[24,25] Apart from the reduction of viscosity, the increased water feed reduced the residence time of the material in the extruder and the conversion of mechanical energy into thermal energy which eventually resulted in the SME reduction.[24]

In the barrel temperature and screw speed interaction effects (Fig. 4(B)), it was found that up to 1200 min^{-1}, SME decreased with increasing barrel temperature and from 1400 min^{-1}, the opposite effect was observed. While increasing the temperature at higher screw speeds, cross-linking effect could overrule the viscosity reduction which required higher mechanical energy for processing. The increase in screw speed produced higher SME both in the 'barrel temperature–screw speed' and 'water feed–screw speed' combinations. The increased shear rate due to increased screw speed was expected to produce higher SME.[26] Extrusion parameters such as high barrel temperature, low water feed and high screw speed levels yielded the highest SME. As mentioned in the torque section earlier, the increased cross-linking (as observed in SEM images) due to the increased screw speed led to the increase in die pressure, torque and SME, which is in contrast to the earlier starch-based studies. In the starch extrusion, the increased screw speed reduces die pressure, torque and SME, respectively, due to the viscosity reduction. But in the protein based raw materials extrusion, the cross-linking effect overrules the viscosity reduction factor which showed opposite effect in our study.

Influence of extrusion parameters on product properties

Influence on colour

The colour measurements are depicted in Fig. 5. The water feed influenced the colour parameters lightness (L^*), b^* values and total colour difference (ΔE) significantly, whereas a^* value was significantly affected by screw speed. It was observed that the higher water feed led to lighter products. The increase in barrel temperature and screw speed caused slight increase in lightness values. The lightness correlates well with ΔE (see also Table 4), as changes in colour mostly occurred by changes in lightness. The increase in water feed reduced the colour difference significantly.

Influence on cooking yield

Figure 6 represents the results with respect to cooking yield. Cooking yield is an indirect measurement of water absorption capacity (WAC) of the extrudates during cooking. Differences in WAC could be explained by both physical and chemical changes occurring in the extrudates. The water binding of proteins is based on two modes of actions namely hydrogen bonding of water and entrapment of water (without drip).[27] The higher the water absorption, the higher is the cooking yield and vice versa. The increase in water feed decreased the cooking yield (Fig. 6(A)). These products exhibited a softer texture; thereby the absorbed water could leak out easily from the softer structure rather than from firmer structure. Oikonomou and Krokida[28] also reported lower WAC with the increased water feed in the protein based raw materials. The increment in barrel temperature decreased the cooking yield up to 55% water feed, whereas the cooking yield increases slightly with increase in barrel temperature for water feed of 60% and more. Lin *et al.*[9] also reported an increased water absorption in soya meat analogues due to the increased temperature in the moisture range of 60 to 70%.

A higher screw speed was found to increase the cooking yield distinctly. Such a higher screw speed can increase the amount of insoluble protein aggregates which could have better water holding capacity (WHC) by entrapping more water rather than soluble protein aggregates.[29–31]

Influence on cutting force

The results with respect to cutting force are shown in Fig. 7. Cutting force of the extrudates was mainly influenced by water feed, whereas barrel temperature and screw speed had minor effects. The effect of barrel temperature was found to be different at low water feed compared to high water feed (from 65%). At lower water feed levels cutting force was found to rise with increase in barrel temperature, whereas a slight reduction in cutting force was observed in products from 65% water feed and more. The latter was in agreement with the study by Lin *et al.*[8] that focused on HME of soya protein.

When the water feed is increased, the cross-linking effect should be reduced due to incomplete protein denaturation and unfolding.[8] The reduced cross-linking resulted in a softer product, thus lower cutting strength was observed. The combination effect of barrel temperature and screw speed did not influence the cutting force distinctly.

In the interaction effects of water feed and screw speed, higher screw speeds increased the cutting force due to the higher cross-linking and polymerization. However, production of smaller structure elements possessing more contact surfaces due to the higher screw speeds also led to higher cutting forces.[32]

Correlation between extruder responses and product properties

Cutting force was correlated positively with die pressure ($r = 0.89$), product temperature ($r = 0.79$), torque ($r = 0.93$) and SME ($r = 0.80$) (Table 4). The positive correlation of SME with cutting force was in agreement with studies by Fang *et al.*[33] and Chen *et al.*[24] where increased tensile strength and hardness of soya meat analogues with increased SME was reported. The torque had negative and positive correlation on L^* ($r = -0.764$) and ΔE (0.769) values, respectively.

The comparison of current study results based on lupin with published studies of HME with soya as raw material revealed some similarities between HME of soya and lupin and the resulting meat analogues. In both our study and a study about soya meat analogues by Lin *et al.*,[9] the extruder barrel temperature was found to have only a small effect on product temperature, die pressure, torque and SME. These similarities can be explained by the fact that in the extrusion of protein rich materials, texturization of protein appears to be the main consequent effect of extrusion heating rather than a lowering of viscosity due to high temperature which is more relevant for starch extrusion. Furthermore, the negative correlation of water feed with all extruder responses were comparable to soya meat analogues.[9] The negative correlation of barrel temperature (in the water feed range of 60 to 65%) and water feed on cutting force of lupin extrudates was also comparable to the findings of soya meat analogues.[8]

Influence on microstructure

The SEM images of the extrudates from selected trials are presented in Fig. 8. In the samples extruded at barrel temperatures of 155 and 180 °C, more aligned layers were formed compared to the product extruded at a barrel temperature of 135 °C. This could be due to the incomplete protein cross-linking reactions at this relatively low temperature. This is in agreement with the study by Chen *et al.*[24] They found that increasing extrusion temperatures from 140 to 160 °C led to increased cross- linking of soya protein. However, only small changes in microstructure of the samples between 155 and 180 °C were found in our investigations.

Regarding the influence of water feed on the microstructure, the extrudate with 55% water feed had long aligned layers, whereas fibrous structure and layers formation were not found in the samples with 40% and 68% water feed. It indicates that either too little (40%) or too much (68%) water feed is not appropriate for the fibrous network structure formation. At less moisture contents, the protein was not completely hydrated in order to participate in the protein cross-linking reactions. At higher moisture contents, the reduced protein denaturation and reduced viscosity of the material inside the extruder could have reduced the protein interactions and cross-linking. Lin *et al*[8] and Liu and Hsieh[34] reported that decreasing the moisture content from 70% to 60% created aligned fibrous structure in soya meat analogues.

The effect of screw speed on the microstructure of the extrudates was also pronounced as observed by the temperature and water feed effects. The increase in screw speed improved the protein texturization, thereby more aligned layers are formed. The sample extruded at a screw speed of 400 $\min^{-1}$ had a rather porous structure, whereas longer layer formation was detected for samples with a screw speed of 1200 and 1800 $\min^{-1}$. The sample extruded with a screw speed of 1800 $\min^{-1}$ had the highest number of layers. Moreover, the denser structure which was observed due to higher screw speeds could have also caused the increase in WHC, thereby increasing cooking yield.

Influence on in vitro *protein digestibility (IVPD)*

The extrusion process significantly improved the IVPD of lupin extrudates compared to that of raw material mixture (Table 5). Despite the reduction of anti-nutritional factors by the extrusion process, the changes in protein structure due to high temperatures and shear appear to be the main influencing factor

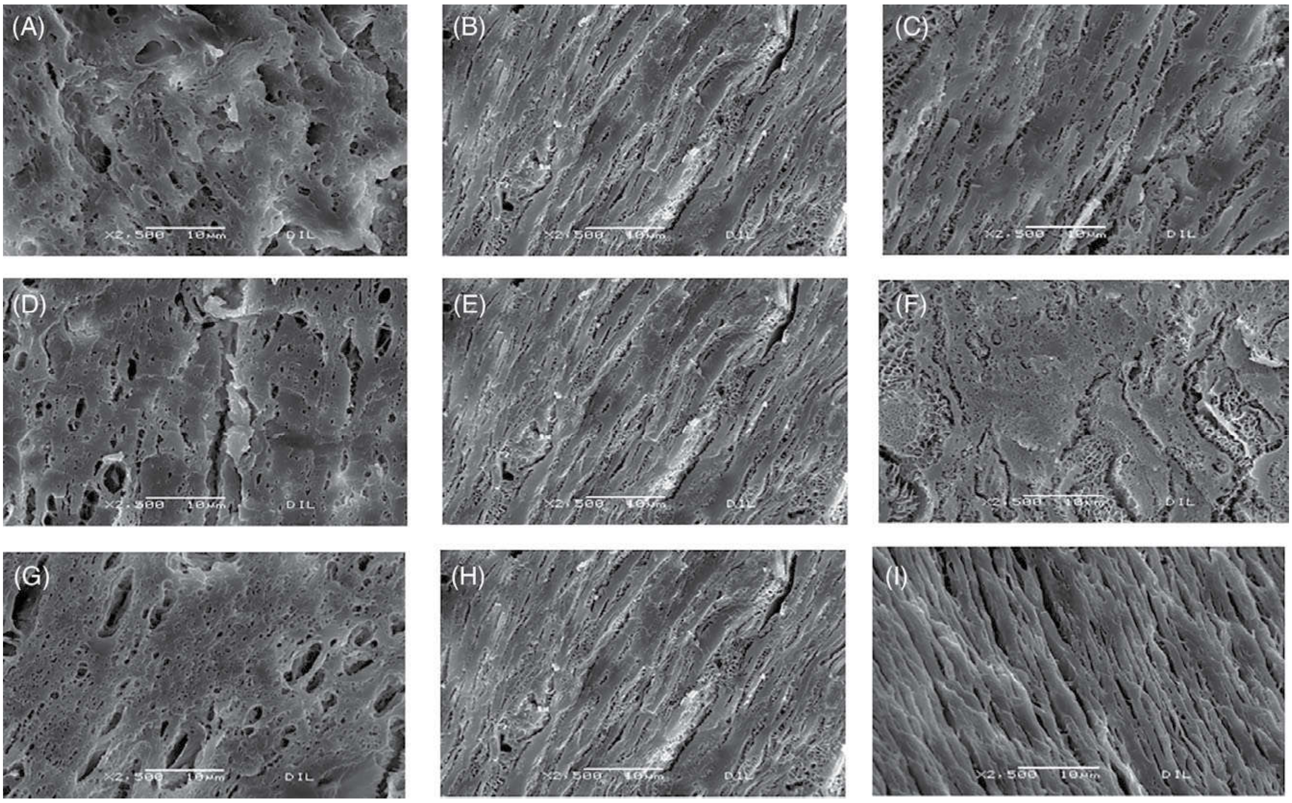

Figure 8. Effect of extrusion parameters on the microstructure of lupin meat analogues (scale bar indicates 10 μm). A (130 °C), B (155 °C) and C (180 °C) show the effect of temperature with the constant water feed (55%) and screw speed (1200 min^{-1}). D (40%), E (55%) and F (68%) show the effect of water feed with the constant temperature (155 °C) and screw speed (1200 min^{-1}). G (400 min^{-1}), H (1200 min^{-1}) and I (1800 min^{-1}) show the effect of screw speed with the constant temperature (155 °C) and water feed (55%).

Table 5. *In vitro* protein digestibility (IVPD) of lupin extrudates[†]

Trial number[‡]	IVPD (%)
Raw material mixture	$78.23 \pm 0.85^{d*}$
3	82.06 ± 0.90^{c}
4	80.94 ± 0.86^{c}
5	85.94 ± 0.79^{a}
9	83.62 ± 0.30^{bc}
15	83.72 ± 0.94^{bc}
16	84.22 ± 0.26^{b}
17	82.68 ± 0.73^{c}

[†]Values are means of triplicates ± standard deviation.
[‡]The corresponding extrusion settings for the trial numbers are listed in Table 1. *Different lowercases mean significantly different values ($P > 0.95$)

for the improvement of protein digestibility.[35] Increasing barrel temperature was found to decrease the IVPD significantly. Higher barrel temperatures could decrease the protein digestibility due to non-enzymatic browning reactions and thermal cross-linking.[36]

Increasing water feed from 40% to 55% did not show significant difference in IVPD, but the sample with 68% water feed showed an increase in IVPD, and it was significantly different compared to 40% and 55% water feed. At higher water feeds (around 60%), protein aggregation was reduced which might enhance protein digestibility.[37] It was found that the screw speed had no significant influence on the IVPD of the extrudates.

CONCLUSION

The HME process showed potential to produce lupin protein-based meat analogues. The results demonstrated that within the wide range of studied extrusion parameters, water feed was the most influencing factor on extruder responses and product properties followed by screw speed, whereas barrel temperature was the least influencing factor. The SEM results showed that the fibrous microstructure of the extrudates was influenced by extrusion parameters. These differences in microstructure are helpful to explain variations in product properties, especially cutting force and cooking yield. The results obtained from this study will help to control the physical and textural characteristics of lupin meat analogues. Compared to the well-established knowledge about HME of soya-based proteins, our results show that similar correlations between extrusion parameters, extruder responses and product properties can be expected for HME of lupin. This means that the long-term experience in soya extrusion will be also useful to produce high quality meat analogues based on lupin protein.

ACKNOWLEDGEMENTS

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4. Publication: Physico-chemical and nutritional properties of meat analogues based on *Spirulina*/lupin protein mixtures

Preface

As a follow up to the previous study, the microalgae "*Spirulina platensis*" was incorporated with lupin protein mixtures for the HME as an alternative protein. For two reasons *Spirulina* was selected in the study: one was to make use of a sustainable protein source for the value addition of *Spirulina*, and the other was to enrich the lupin meat analogues more nutritionally with antioxidant properties.

The effect of different extrusion conditions (temperature/water feed content/screw speed) on physicochemical properties of the lupin/*Spirulina* based meat analogues was evaluated. The antioxidant properties of the extrudates were also additionally evaluated. As mentioned in the literature, only the knowledge on starch based dry extruded products is available with regard to antioxidant properties. Hence, it is interesting to know how the antioxidant properties behave in the HME environment unlike in low moisture conditions, which were already explored. Furthermore, FTIR analyses of the extrudates were conducted to evaluate the secondary structural changes of protein after extrusion with the aim of understanding the relationship between protein structural changes and physicochemical properties of extrudates.

In this study, Stefan Töpfl, Ralf G. Berger and Christian Hertel contributed project ideas and were involved in the manuscript preparation process.

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ORIGINAL PAPER

Physico-chemical and nutritional properties of meat analogues based on *Spirulina*/lupin protein mixtures

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Abstract

The effect of the addition of *Spirulina platensis* flour and of extrusion parameters on texture, cooking yield, expressible moisture, total phenolic content (TPC), total flavonoid content (TFC), Trolox equivalent antioxidant activity (TEAC), in vitro protein digestibility (IVPD) and conformational changes of proteins using Fourier-transform infrared spectroscopy (FTIR) of lupin protein based meat analogues was studied. High moisture extrusion (HME) cooking was used to produce the meat analogues. The *Spirulina* concentration (15, 30 and 50%), extruder barrel temperature (145 °C, 160 °C and 170 °C), water feed (50, 55 and 60%), and screw speed (500, 800 and 1200 rpm) were varied. The *Spirulina* concentration and extrusion parameters significantly affected physical properties, such as texture, cooking yield and expressible moisture of the extrudates. The addition of *Spirulina* generally increased the TPC, TFC and TEAC values of the extrudates. Increased temperature and screw speed as well as decreased water feed slightly improved the content of TPC, TFC and TEAC, respectively. The addition of *Spirulina* at a level of 30% decreased the IVPD of the extrudates from 82 to 75.6%. However, increased water feed and screw speed partly counterbalanced this effect. Protein conformational analyses of the extrudates by FTIR showed that β-sheets were decreased, whereas α-helix, β-turn and antiparallel β-sheets were increased compared to the raw extrusion mixtures. On the whole, the HME process improved the values of TPC, TFC, TEAC and IVPD in the extrudates compared to the raw extrusion mixtures. The addition of *Spirulina* along with controlled extrusion parameters can deliver meat analogues with improved physico-chemical and nutritional properties.

Keywords Spirulina · High moisture extrusion · Lupin · Sustainability

Introduction

According to FAO figures, the world population is expected to reach 9 billion by 2050 with a concomitant food production increase by 70%. In addition to growing population, urbanisation and growing economy in the developing countries led to an increased consumption of animal protein over past decades. If the current global meat consumption trend continues, the animal based protein demand will be doubled by 2050, which is a serious concern in terms of sustainability [1, 2]. The sustainability issues with animal proteins are linked to increasing feed supplies and higher levels of greenhouse gases production [3]. Therefore, the need to search for sustainable alternative proteins to replace animal protein partially became unavoidable. Microalgae can be one of the alternative protein sources, which would potentially resolve the sustainability issues, as high productivity of microalgae with high protein concentration can be achieved with potential low use of land and water [4].

Microalgae have been used as food by humans for thousands of years [5], and are rich in proteins, long-chain polyunsaturated fatty acids, carotenoids, vitamins, minerals, phenolics as well as other bioactive molecules [6]. More than 50,000 microalgal species are reported to exist in the world and among them the number of species studied are only 30,000 [7]. The genera of commercial importance are *Spirulina*, *Chlorella*, *Haematococcus*,

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Dunaliella, *Botryococcus*, *Phaeodactylum*, *Porphyridium*, *Chaetoceros*, *Crypthecodinium*, *Isochrysis*, *Nannochloris*, *Nitzschia*, *Schizochytrium*, *Tetraselmis*, and *Skeletonema*. In the current market, *Spirulina* (*Arthrospira*) and *Chlorella* are most dominant [8]. Microalgae are available in the health food sector in the forms of tablets, capsules and liquids [9]. They are incorporated into products such as snacks, candy, gums, noodles, wine, beverages, and breakfast cereals [10–12]. *Spirulina platensis* (*Arthrospira platensis*) (hereafter mentioned as *Spirulina*) used in this study is a prokaryotic cyanobacterium and plays an important role in the regulation of the planetary biosphere [13]. Although *Spirulina* contains many nutrients, the high protein concentration is of particular interest for the development of protein-rich foods.

Meat analogues are commonly produced from protein sources like soy, wheat, pea, milk, egg and fungal substrates by applying the high moisture extrusion (HME) technology. Such products are expected to have texture, mouthfeeling, taste, and nutritional value similar to those of meat products [14]. The protein-based raw materials are melted at high temperature and pressure during the HME process, and the melt is aligned to create a meat like structure using the cooling die at the end of the extruder [15]. Few studies worked on the incorporation of *Spirulina* in extruded snacks using dry extrusion [16–18]. However, own preliminary data showed that HME processed *Spirulina* biomass results in products lacking the fibrous structure (data not shown). Therefore, the blending of *Spirulina* biomass with other plant proteins possessing texturization functionality is mandatory. Grahl et al. [19] reported the effect of addition of *Spirulina* and extrusion parameters on the sensory properties of HME-based soya meat analogues. However, there are no data on blending of spirulina biomass with other plant proteins. In addition, to broaden the value addition of spirulina biomass and the varieties of meat analogues, other plant protein sources should be considered.

Due to the predominate usage of soya bean for food and feed in Europe, 70% of soya bean must be imported. The sweet lupin varieties such as White lupin (*Lupinus albus* L.), yellow lupin (*L. luteus* L.) and narrow-leafed lupin (*L. angustifolius* L.) are native to Europe and can be an alternative to soya bean. The sweet lupin varieties are not bitter due to the absence of alkaloids [20]. Palanisamy et al. [21] already reported the feasibility of lupin protein (*L. angustifolius* L.) for the meat analogues production. Hence, in this follow-up study, *Spirulina* biomass was used along with lupin to increase the value addition of *Spirulina* biomass. The objective of this study was to investigate the effect of extrusion parameters on the texture, cooking yield, expressible moisture, total phenolic concentration (TPC), total flavonoid concentration (TFC), trolox equivalent antioxidant activity (TEAC) and protein conformational changes of meat analogues produced by HME using a combination of lupin and *Spirulina* as protein sources.

Materials and methods

Raw materials

Lupin protein isolate (produced in Peru) of the narrow-leafed lupin *(L. angustifolius* L.) was purchased from Wellness & Healthcare Service GmbH, Neuenbürg, Germany. Lupin protein concentrate was procured from Frank Food Products, Twello, Netherlands. The dried *Spirulina* biomass, cultivated in open raceway ponds in Asia, was purchased from the Institute for Food and Environmental Research (ILU e.V.) in Nuthetal, Germany. The proximate composition, such as moisture, protein, fat and ash concentration of *Spirulina* biomass, lupin protein concentrate and isolate was analyzed according to German official methods (§ 64 LFGB). The carbohydrate concentration was calculated by the difference method.

High moisture extrusion (HME)

HME process was performed using a Coperion ZSK 27 Mv PLUS twin screw extruder (Coperion GmbH, Stuttgart, Germany). The *Spirulina* biomass was mixed with lupin protein mixture and iota carrageenan using a Stephan UMC 12 universal machine (STEPHAN food service equipment GmbH, Hameln, Germany). The compositions used in HME are listed in Table 1. The chosen ratio of lupin concentrate and lupin isolate and extrusion conditions were based on the data obtained from the preliminary trials (data not shown). The variable working parameters used for HME were: *Spirulina* concentration (15, 30 and 50%), temperature (145, 160 and 175 °C), water feed (50, 55 and 60%) and screw speed (500, 800 and 1200 rpm). To investigate the effect of one parameter (spirulina concentration, temperature, water feed or screw speed), the other remaining parameters were kept constant as follows: 30% *Spirulina* concentration, 160 °C temperature, 55% water feed or 800 rpm screw speed. In Table 2 the conditions applied to the different HME trials are compiled.

Table 1 Concentration of ingredients in the recipes for HME

Ingredient	Concentration (%) in recipe			
	1	2	3	4
Lupin protein isolate	67.9	57.4	46.9	32.9
Lupin protein concentrate	29.1	24.6	20.1	14.1
Spirulina biomass	–	15.0	30.0	50.0
Iota (i) carrageenan	3.0	3.0	3.0	3.0

Table 2 Conditions used for HME trials

Trials	Recipe no.	Temperature (°C)	Water feed (%)	Screw speed (rpm)
1	1	160	55	800
2	2	160	55	800
3	3	160	55	800
4	4	160	55	800
5	3	145	55	800
6	3	175	55	800
7	3	160	50	800
8	3	160	60	800
9	3	160	55	500
10	3	160	55	1200

The extruder barrel consisted of totally nine heating zones. During the trials, temperatures of zones 1, 2, 3, 4, 8 and 9 were kept constant and set as 40, 60, 90, 120, 140 and 120 °C, respectively. To study the effect of temperature, only barrel temperatures of zones 5, 6 and 7 were modified for the trials. The total throughput was set to 8 kg h^{-1}. At the exit of the extruder barrel, a long cooling die was attached, where the laminar flow of the extruded mass occurred and texturization took place. The cooling die was maintained at the temperature of 30 °C using a cooling bath. The cooling die had internal dimensions of 50 × 15 × 800 mm (W × H × L). In between the trials, the process was allowed to run stable for 10 min, and then the samples were taken, vacuum packed and stored at -20 °C for the analyses.

Cutting force

The cutting force of the extrudates was measured using a Texture Analyser (Stable Micro Systems TA XT 2, Surrey England) according to Palanisamy et al. [22]. Before the measurement, the system was calibrated using a 5 kg cell. The frozen samples were thawed overnight in the refrigerator and left in room temperature for 2 h before the measurement. The thawed samples were cut into the size of 20 × 20 × 15 mm (L × W × H) and in the texture analyser, the sample pieces were cut using a razor blade with a pre-test speed of 1.0 mm s^{-1}, a test speed of 2.0 mm s^{-1} and a post-test speed of 10 mm s^{-1}. The samples were cut in the transverse direction of the die exit, and the maximum force was used as the measure for cutting force. The cutting force in the longitudinal direction was not measured, as it showed the same trend as transverse direction in the earlier study [22]. Five measurements were taken for each sample.

Cooking yield

The cooking yield was determined according to the method of Palanisamy et al. [22]. The extrudates were thawed and cut as mentioned above. The cut samples which had pH value of around 6.5 were directly cooked in water for 20 min at 80 °C. The cooking conditions were chosen based on data obtained from preliminary trials (data not shown). After 20 min, the samples were removed from the water and the excess water was drained using a sieve and the samples were left at room temperature for 10 min to evaporate the surface water completely. The mass before and after heating was taken and cooking yield was calculated using Eq. (1). The analysis was performed in triplicate,

$$\text{Cooking yeild} = \frac{m_{CS}}{m_{RS}} \times 100\%, \tag{1}$$

m_{RS} and m_{CS} are the mass of the sample before and after cooking, respectively.

Expressible moisture

Expressible moisture was analysed using the modified Hamm procedure [23]. Approximately 1 g of cooked meat analogue was taken from the cooking yield experiments and placed between two filter papers and kept under a manual press which weighed around 10 kg for 2 min. Mass of the sample was taken before and after the pressing, and expressible moisture was expressed as a percentage of the net mass difference from the initial mass Eq. (2).

$$\text{Expressible moisture (\%)} = (\text{initial mass} - \text{squeezed mass}) / (\text{initial mass}) \times 100. \tag{2}$$

Sample extraction for total phenolics concentration (TPC), total flavonoids concentration (TFC) and trolox equivalent antioxidant capacity (TEAC)

The frozen extrudates were freeze dried and ground passing through a 0.5 mm sieve in a centrifugal mill type ZM1 from Retsch (Haan, Germany). The moisture content of the freeze-dried samples was around 5%, whereas the raw extrusion mixtures contained around 6.5%. The weight of the samples taken for the extraction was adjusted according to their moisture concentration. The sample extraction for TPC, TFC and TEAC was performed according to Martínez-Villaluenga et al. [24]. Around 1 g of ground sample was mixed with 10 ml of 80% methanol and shook using a shaking incubator at 500 rpm for 2 h. After that, the sample mixture was centrifuged at 20 °C, 10000 rpm for 15 min. The supernatants of the centrifuged mixtures were used for analyzing TPC, TFC and TEAC of the raw extrusion mixtures and extrudates.

Total phenolics concentration (TPC)

The TPC of raw extrusion mixtures and extrudates were analysed using a modified version of the Folin–Ciocalteu assay [25]. Gallic acid was used as a standard. The 20 µL of supernatant extracts were mixed with 1.58 mL distilled water and then Folin–Ciocalteu reagent (100 µL). After the waiting time of between 30 s and 8 min, 300 µL of 20% sodium carbonate was added to the mixture. Immediately the samples were vortexed and incubated for 2 h in the dark at room temperature. The absorbance was measured at 765 nm using Gene Quant 1300 UV–Vis spectrophotometer. The samples and standards were analysed in triplicate. The TPC was expressed as mg GAE (gallic acid equivalent) g^{-1} sample weight (dry mass).

Total flavonoids concentration (TFC)

TFC was determined according to the method described by Jia et al. [26]. Known concentrations of catechin were used as standards. Firstly, 0.25 ml of 80% methanolic extract was added to 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. The mixture was left at room temperature for 6 min. After that, 150 µl of 10% $AlCl_3 \times 6\ H_2O$ solution was added and the mixture was allowed to stand at room temperature for further 5 min. After the resting time, 0.5 ml of 1 M NaOH was added and thoroughly mixed before measuring the absorbance at 510 nm using Gene Quant 1300 UV–Vis spectrophotometer. All the samples and standards were analyzed in triplicate. Total flavonoids concentration was expressed as mg CE (catechin equivalent) g^{-1} sample weight (dry mass).

Determination of Trolox equivalent antioxidant capacity (TEAC)

The TEAC of the sample extracts was determined according to Re et al. [27]. For the stock solution, 7 mM of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was prepared with 2.45 mM potassium persulfate and left in the dark for around 16 h. This stock solution was diluted with 80% methanol until it reached an absorbance of 0.70 ± 0.02 at 734 nm. Afterwards 2 ml of ABTS cation solution was added either with 20 µl of the sample extracts or Trolox standards, and the absorbance was measured at 734 nm using a Gene Quant 1300 UV–Vis spectrophotometer. The analysis was conducted in triplicate, and the results were expressed as µl TE (Trolox equivalent) g^{-1} of sample dry weight.

In vitro protein digestibility (IVPD)

In vitro protein digestibility (IVPD) of freeze dried extrudates was determined by the modified method of Hsu et al. [28] as described by Tinu et al. [29]. Freeze-dried extrudates were ground as mentioned earlier. Sample weight equivalent to 62.5 mg protein was taken, suspended in 10 ml of distilled water and was incubated at 37 °C for 1 h in a shaking incubator. Then, the pH of the suspension was adjusted to 8.0 using 0.1 M NaOH and/or 0.1 M HCl. To prepare the multi-enzyme solution, 16 mg trypsin (T0303 Trypsin from porcine pancreas Type IX-S, lyophilized powder, 13,000–20,000 BAEE units mg^{-1} protein), 31 mg of chymotrypsin (C4129 a-Chymotrypsin from bovine pancreas C4129 Type II, lyophilized powder, P40 units mg^{-1} protein) and 13 mg protease (P5147 Protease from *Streptomyces griseus* Type XIV, P3.5 units mg^{-1} solids) were diluted in 10 ml of distilled water. Protease from *S. griseus* was used in this study, since the peptidase applied in the original method (Sigma P7500 Peptidase from porcine intestinal mucosa, 50–100 units g^{-1} solids) was no longer available [29]. The enzyme solution was kept at 37 °C, while the pH was adjusted to 8.0 as described above. Then 1 ml of multi-enzyme solution was added to 10 ml of sample suspension. The pH value of this blend was measured immediately after addition of multi-enzyme solution (pH_0) and after 10 min (pH_{10}). Equation (3) below was used to calculate the IVPD. The analysis was conducted in triplicate.

$$\mathrm{IVPD} = 65.66 + 18.10(\mathrm{pH0} - \mathrm{pH10}). \tag{3}$$

Fourier-transform infrared spectroscopy (FTIR)

The freeze dried and powdered samples were analysed under vacuum using Fourier transform infrared spectroscopy (Bruker Vertex 80 V). The spectra were recorded between 5000 and 370 cm^{-1} with an average of 32 scans with a resolution of 2 cm^{-1}. Few of the samples were run in triplicate to check the reproducibility of the spectra and deconvoluted data (data not shown). Since the reproducibility was precise in multiple runs, thereafter a single run was performed for all of the samples. Deconvolution of the amide I bands using Lorentzian functions in one step without any baseline correction was performed (1600–1700 cm^{-1}) using the Curve fit program of OPUS 7.5. The integral of each peak was divided by the sum of all peaks in the amide I area to calculate the proportion of each structure in the amide I area. The deconvoluted peaks in the amide I area were assigned to β-sheets (1615–1637 cm^{-1}), α-helix (1648–1660 cm^{-1}), β-turn and loops (1662–1678 cm⁻1) and antiparallel β-sheets (1682–1690 cm⁻1), respectively [30, 31].

Table 3 Proximate composition of the raw materials

Parameter	Content (%)		
	Lupin protein isolate	Lupin protein concentrate	*Spirulina* biomass
Moisture	5.51	6.87	5.29
Protein	77.80	51.10	68.50
Fat	3.73	9.94	6.40
Ash	4.98	3.19	6.30
Carbohydrate[a]	7.98	28.90	13.50

[a]Calculated by difference

Fig. 1 HME processed extrudates

Statistical analysis

One way Analysis of Variance (ANOVA) test was performed to evaluate the significance (significance level at $p < 0.05$) between the samples using the statistical programme DataStar 3.4.

Results and discussion

The proximate compositions of the raw materials used are listed in Table 3. The lupin protein isolate and concentrate differed markedly in composition. According to the concentrations of *Spirulina* biomass (15–50%) used in the recipes, the amount of lupin protein isolate and concentrate was adjusted (Table 1). Furthermore, iota carrageenan was added to the mixtures, as preliminary experiments showed that the addition of 3% iota carrageenan improved texturization of lupin protein and *Spirulina* biomass mixtures (data not shown). In summary, ten different meat analogues with 15, 30 or 50% *Spirulina* biomass mixed with lupin proteins were produced using HME by altering the parameters temperature, water feed and screw speed (Table 2). With increasing *Spirulina* concentration the colour of the extrudates turned to dark green (Fig. 1). The occurrence of dark colour could be due to the process oriented partial degradation of *Spirulina* pigments like phycocyanin, carotenoids and chlorophyll [17].

Table 4 Effect of recipe components and extrusion parameters on the physical properties of extrudates

Samples	Cutting force (N)	Cooking yield (%)	Expressible moisture (%)
Extrudates[a] with *Spirulina* content of			
0%	8.91 ± 0.55^{b}	115.90 ± 0.91^{a}	2.62 ± 0.59^{c}
15%	8.62 ± 0.42^{b}	116.00 ± 0.75^{a}	4.51 ± 0.85^{b}
30%	6.98 ± 0.75^{cd}	112.80 ± 1.03^{b}	3.85 ± 0.61^{b}
50%	5.96 ± 0.63^{d}	110.20 ± 1.46^{bc}	3.56 ± 0.97^{bc}
Extrudates with 30% *Spirulina* produced at temperature of			
145 °C	6.46 ± 0.6^{d}	110.10 ± 0.54^{c}	3.94 ± 1.52^{bc}
160 °C	6.98 ± 0.75^{cd}	112.80 ± 1.03^{b}	3.85 ± 0.61^{b}
175 °C	7.67 ± 0.21^{c}	112.00 ± 0.75^{b}	2.25 ± 1.14^{bc}
Extrudates with 30% *Spirulina* produced with water feed of			
50%	11.41 ± 0.46^{a}	116.30 ± 0.91^{a}	3.23 ± 0.58^{bc}
55%	6.98 ± 0.75^{cd}	112.80 ± 1.03^{b}	3.85 ± 0.61^{b}
60%	4.23 ± 0.38^{e}	110.20 ± 0.69^{c}	12.05 ± 2.81^{a}
Extrudates with 30% *Spirulina* produced with screw speed of			
500 rpm	6.47 ± 0.33^{d}	112.30 ± 1.17^{bc}	3.47 ± 0.68^{bc}
800 rpm	6.98 ± 0.75^{cd}	112.80 ± 1.03^{b}	3.85 ± 0.61^{b}
1200 rpm	7.71 ± 0.70^{c}	115.30 ± 0.25^{a}	2.65 ± 0.07^{c}

Means within the same column with different superscripts are significantly different ($P < 0.05$)

[a]Produced with constant extrusion temperature (160 °C), water feed (55%) and screw speed (800 rpm)

Physical properties of meat analogues

Cutting force

Cutting force indicates the indirect measurement of hardness and fibrous structure formation or degree of texturization [32]. The results of cutting force are shown in Table 4. The addition of *Spirulina* at levels of 30% and 50% significantly decreased the cutting force compared to the control. This indicated that the fibrous structure formation is decreased by increasing the addition of *Spirulina* biomass. The increase in temperature from 145 to 160 °C had no significant effect on the cutting force, but at 175 °C there was a significant increase. This increased cutting force might be caused by higher protein denaturation at higher temperature. Lin et al. [33] showed that the increase in water feed can result in incomplete protein denaturation and unfolding which lead to decreased protein texturization and cutting force. Changes in the water feed had a great impact on the cutting force, even though the water feed was in a small range (50–60%). This indicates that the water concentration is a crucial parameter for texturization. The increase in screw speed from 500 to 1200 rpm significantly increased the cutting force of meat analogues. In addition to the increased texturization due to the higher screw speed, production of small structural elements in the extrudates could also have led to increased cutting forces [34]. The effect of extrusion temperature, water feed and screw speed on cutting force is in agreement with the study by Grahl et al. [19] which focused on the meat analogues produced from the mixtures of soya protein concentrate and *Spirulina* biomass. Both hardness and degree of fibrousness/texturization can influence the sensory acceptance of the meat analogues. In the current study, the cutting force of the different meat analogues ranged from 4.23 to 11.41 N. Palanisamy et al. [22] found that among the soya meat analogues for which the cutting force ranged from 7.85 to 10.39 N, extrudates with a cutting force of approximately 9.26 N are sensorically acceptable. Texturization might be better with higher cutting force, however, a too hard product might affect the sensorial acceptance. So, it is important to consider both hardness and texturization properties for the right selection the extrusion parameters.

Cooking yield

Cooking yield is an important parameter considering not only the economic aspects in food industries, but also the amount of water absorbed during cooking will also influence the sensorial properties, for example. juiciness. Products with high *Spirulina* concentration (30 and 50%) showed lower cooking yield than the control without *Spirulina* (Table 4). Remarkably, the use of a low *Spirulina* concentration, increased temperature, increased screw speed and decreased water feed in general yielded harder products, exhibiting higher cutting force and cooking yield. This indicated that products with hard structures were able to hold the water with low drip loss better than soft structured products. It is tempting to speculate that the use of increased temperature, increased screw speed and decreased water feed in the extrusion process can increase the amount of insoluble protein aggregates. Other authors found that higher concentrations of insoluble protein aggregates contributed to a higher water holding capacity due to the better water entrapment between the protein network structure [35, 36, 37] which could explain the increased cooking yield of the extrudates.

Expressible moisture

The estimation of expressible moisture is an indirect measurement of water holding capacity which can influence the sensorial properties and further processing steps required to make end products from extrudates (e.g. nuggets). The lupin protein extrudate exhibited less water loss after pressing than the *Spirulina* added extrudates (Table 4). Extrusion temperature had no significant effect on the water loss unlike water feed and screw speed. The extrudate produced with 60% water feed showed the highest water loss, which was significantly different compared to all the other meat analogues. The low cutting force observed with this sample indicated that the water cannot be retained in such a soft structure upon pressing, as also found in the samples produced at lower screw speeds. In the protein-based systems, compact structure holds the water better than the soft structure [22]. Hence, hardness property of the extrudate highly influenced both the cooking yield and expressible moisture.

Total phenolics concentration (TPC), total flavonoids concentration (TFC) and Trolox equivalent antioxidant capacity (TEAC)

Phenolic compounds comprise simple phenols, flavonoids, phenylpropanoids, tannins, lignins, phenolic acids and their derivatives, which are synthesized as secondary plant metabolites [38]. They have gained attention due to their presumed health benefits as natural antioxidants [39]. TPC, TFC and antioxidant activity of protein based meat analogues were not yet researched in detail. The concentrations are shown in Table 5. The higher the amount of *Spirulina*, the higher the TPC, TFC and TEAC values were observed in the mixtures and extrudates. In previous studies, the addition of *Spirulina* also improved the concentration of phenolics and antioxidant activity in products, such as pasta and cookies [40, 41].

Remarkably, the extrusion process improved the TPC and TFC in the extrudates compared to the raw extrusion mixtures, which was consistent with the increased TEAC of the extrudates. The increased TPC and TFC concentrations

Table 5 Total phenolic concentration (TPC), total flavonoid concentration (TFC), Trolox equivalent antioxidant activity (TEAC) and in vitro protein digestibility (IVPD) of raw extrusion mixtures and extrudates

Samples	TPC (mg GAE g^{-1} dm)	TFC (mg CE g^{-1} dm)	TEAC (µmol TE g^{-1} dm)	IVPD (%)
Raw extrusion mixtures with *Spirulina* content of				
0%	1.90 ± 0.02^{j}	0.79 ± 0.06^{h}	5.53 ± 1.03^{f}	75.19 ± 1.01^{c}
15%	3.33 ± 0.44^{h}	1.03 ± 0.11^{g}	6.09 ± 0.88^{ef}	72.12 ± 0.87^{d}
30%	4.89 ± 0.50^{f}	1.19 ± 0.07^{f}	6.64 ± 0.32^{e}	71.04 ± 1.18^{d}
50%	6.91 ± 0.38^{b}	1.42 ± 0.13^{cde}	7.58 ± 0.49^{d}	69.63 ± 1.51^{d}
Extrudates[a] with *Spirulina* content of				
0%	1.94 ± 0.04^{i}	0.77 ± 0.04^{h}	5.92 ± 0.34^{f}	82.04 ± 0.94^{a}
15%	4.38 ± 0.45^{g}	1.24 ± 0.02^{f}	7.71 ± 1.54^{cde}	78.19 ± 1.02^{b}
30%	5.59 ± 0.30^{d}	1.51 ± 0.02^{c}	8.98 ± 0.34^{b}	75.61 ± 0.63^{c}
50%	8.47 ± 0.70^{a}	1.83 ± 0.03^{a}	9.99 ± 0.49^{a}	73.70 ± 1.08^{cd}
Extrudates with 30% *Spirulina* produced at temperature of				
145 °C	5.38 ± 0.70^{def}	1.37 ± 0.05^{e}	8.55 ± 0.39^{c}	75.42 ± 1.15^{c}
160 °C	5.59 ± 0.30^{d}	1.51 ± 0.02^{c}	8.98 ± 0.34^{b}	75.61 ± 0.63^{c}
175 °C	5.57 ± 0.45^{de}	1.44 ± 0.03^{d}	9.18 ± 0.36^{b}	73.20 ± 2.64^{cd}
Extrudates with 30% *Spirulina* produced with water feed of				
50%	5.72 ± 0.45^{cd}	1.65 ± 0.05^{b}	9.13 ± 0.93^{bc}	76.52 ± 0.77^{bc}
55%	5.59 ± 0.30^{d}	1.51 ± 0.02^{c}	8.98 ± 0.34^{b}	75.61 ± 0.63^{c}
60%	5.29 ± 0.21^{e}	1.32 ± 0.06^{e}	8.87 ± 0.29^{bc}	76.87 ± 1.85^{bc}
Extrudates with 30% *Spirulina* produced with screw speed of				
500 rpm	5.36 ± 0.54^{def}	1.40 ± 0.07^{de}	8.99 ± 0.38^{b}	76.57 ± 1.33^{bc}
800 rpm	5.59 ± 0.30^{d}	1.51 ± 0.02^{c}	8.98 ± 0.34^{b}	75.61 ± 0.63^{c}
1200 rpm	6.10 ± 0.52^{c}	1.51 ± 0.03^{c}	9.09 ± 0.88^{bc}	77.08 ± 0.79^{bc}

Means within the same column with different superscripts are significantly different ($P < 0.05$)

[a]Produced with constant extrusion temperature (160 °C), water feed (55%) and screw speed (800 rpm)

are attributed to the disintegration of the cell wall of the raw material, which facilitated the extraction of phenolic compounds [42].

The modification of the temperature did not significantly influence the TPC of the extrudates. Increasing the water feed from 50 to 60% significantly decreased the TPC, while an increase in screw speed from 500 to 1200 rpm increased the TPC concentration significantly. The decrease in phenolic concentration due to the increased water feed may be explained by an increased polymerization of the phenolic compounds. Thereby the extractability of phenolics may have been reduced [43].

At higher screw speed and lower water feed, higher concentration of phenolic compounds were observed. The higher screw speed may have imposed an increased shear stress on the cell wall [44], thereby resulting in a better extraction of phenolic compounds. The control extrudate showed no significant differences of TFC and TEAC compared to the raw extrusion mixture, but the extrudates with *Spirulina* had higher TFC and TEAC than the mixtures. Increased temperature from 145 to 160 °C led to a higher concentration of TFC and increased the TEAC, whereas increasing the temperature to 175 °C decreased the TFC significantly, but no significant differences were found in the TEAC between 160 and 175 °C. There was a significant reduction in TFC with an increased water feed. An increase in TFC was also observed with an increase in screw speed from 500 to 1200 rpm, but without significant differences between 800 rpm and 1200 rpm. The increase in phenolic and flavonoids in the extrudates resulted in an increased TEAC. Apart from this, some Maillard reaction products may have resulted in an increase of the TEAC [44, 45]. Few studies reported also increased antioxidant activity at elevated extrusion temperature [46, 47].

Several cereal based extrusion studies reported that the extrusion process decreased the TPC, TFC and antioxidant activity [46, 48], while others stated the opposite [49, 50]. The extrudate with 50% *Spirulina* had the highest antioxidant activity. Hence, depending on the raw material and extrusion conditions used, the extrusion process can be either positive or negative in terms of TPC, TFC and antioxidant activity.

In-vitro protein digestibility (IVPD)

The extrusion process can cause changes in the secondary, tertiary and quaternary structures of proteins, thereby, proteolytic enzyme gain better access and open peptide bonds, which eventually improves the protein digestibility (PD) [51]. Only the raw extrusion mixture with lupin proteins showed increased PD compared to the *Spirulina* incorporated extrusion mixture (Table 5). It was found that all the extrudates exhibited a higher PD than the raw extrusion mixtures except the 50% *Spirulina* added extrudate. No significant difference existed between the 50% *Spirulina* added mixture and the extrudate. By increasing the amount of *Spirulina*, the PD values were decreased both in the mixtures and the extrudates. The cell wall components of the microalgae biomass may be involved in the decrease of the PD. Even though the *Spirulina* cell wall is relatively thin with a thickness of 40–60 nm [52], this cell wall layer could have influenced the PD negatively in comparison to the mixtures and extrudates of pure lupin protein, where no cell walls exist. The modifications of extrusion temperature, water feed and screw speed had no significant effect on the PD.

Fourier-transform infrared spectroscopy (FTIR)

The changes in the secondary structure of proteins in HME processed meat analogues were not yet studied. An example of original spectra and a typical deconvoluted FTIR spectrum are provided in the supplementary material. α- and β-structures can be either increased or decreased after the extrusion process depending on the type of protein and extrusion conditions (temperature, water feed and screw speed). In the extrudates, β-sheets were decreased, whereas α-helix, β-turn and antiparallel β-sheets were increased compared to the raw extrusion mixtures (Table 6). The decrease in β-sheets and increase in α-helix was in agreement with the results obtained by Zhou et al. [53] for the extrusion of rice bran protein. The increase in *β*-turn and antiparallel β-sheets was in agreement with a study on extruded pea protein isolate [54].

Table 6 Percentage of amide I components in the raw extrusion mixtures and extrudates

Samples	β-Sheets (%)	α-Helix (%)	β-Turn/loops (%)	Antiparallel β-sheets (%)
Raw extrusion mixtures with *Spirulina* content of				
0%	62.11	29.91	5.55	2.43
15%	63.96	24.77	8.01	3.26
30%	63.73	25.79	7.51	2.97
50%	63.35	27.19	6.92	2.54
Extrudates[a] with *Spirulina* content of				
0%	51.40	38.34	7.43	2.83
15%	55.25	29.79	11.24	3.72
30%	51.48	33.89	11.30	3.33
50%	47.62	38.39	11.14	2.86
Extrudates with 30% *Spirulina* produced at temperature of				
145 °C	49.35	28.51	13.90	6.29
160 °C	51.48	33.89	11.30	3.33
175 °C	53.53	32.13	11.00	3.34
Extrudates with 30% *Spirulina* produced with water feed of				
50%	48.07	38.85	10.49	2.60
55%	51.48	33.89	11.30	3.33
60%	50.90	36.61	10.06	2.43
Extrudates with 30% *Spirulina* produced with screw speed of				
500 rpm	52.64	31.81	11.75	3.81
800 rpm	51.48	33.89	11.30	3.33
1200 rpm	53.96	32.19	10.67	3.18

[a]Produced with constant extrusion temperature (160 °C), water feed (55%) and screw speed (800 rpm)

The addition of *Spirulina* had a clear impact on the secondary structure of proteins (Table 6). With an increased addition of *Spirulina*, β-sheet values were decreased, whereas α-helix values had an increasing trend. While increasing the temperature, β-sheets were increased, whereas β-turns and antiparallel β-sheets had a decreasing trend and α-helix values showed an unclear trend. The modification of water feed did not show a clear effect on all of the amide I components. β-turn and antiparallel β-sheet values were decreased with increasing the screw speed, whereas β-sheet and α-helix values did not show a clear trend.

The changes, which occurred in the amide-I area due to the modification of extrusion parameters were smaller than the differences between the mixtures and extrudates. This could explain why the IVPD between mixtures and extrudates were significantly different, but not between the samples with modified extrusion parameters (see Table 5).

The higher values for the β-sheets are known to be negatively correlated with PD, as the peptidase activity can be hindered by the presence of a large number of hydrogen bonds between β-sheets [55]. Carbonaro et al. [56] found that a decrease in β-structures improved the PD in dairy products and legumes including white common bean, chickpea and soybean. The present FTIR results showed that the β-sheets were reduced after the extrusion, which could also explain the increased PD in the extrudates (Table 5). According to Bai et al, higher values of α-helices and loops/β-turns are positively correlated with IVPD, which was also observed in this study. Due to the strong and flexible structure of loops and presence of fewer hydrogen bonds, the enzyme access to the protein is facilitated and the PD is improved accordingly [55]. No clear relationships were found between the secondary structural changes and physical properties such as cutting force, cooking yield and expressible moisture.

Conclusion

Spirulina flour can be used up to 50% with lupin protein to produce HME-based meat analogues. Desirable physical properties of the meat analogues can be achieved by adjusting the extrusion parameters, such as temperature, water feed and screw speed. The antioxidant activity and in vitro protein digestibility (IVPD) were slightly improved by appropriate extrusion parameters. The observed conformational changes of proteins in the extrudates were positively linked to the IVPD. The improvement in protein digestibility and antioxidant properties of *Spirulina* incorporated meat analogues is regarded beneficial for the human diet. In vivo studies should be conducted to confirm the bioavailability of antioxidants and protein digestibility of HME based *Spirulina* foods. Unlike the regular end products manufactured from meat analogues, such as nuggets, the intermediate *Spirulina* incorporated meat analogues will open new opportunities. Products, such as sushi, protein-rich salad, jerky and ravioli (as a filling) type products will additionally benefit from the dark green color of the extruded proteins. The increase in protein digestibility and antioxidant activity due to the high moisture extrusion process can open a gateway for other alternative proteins for developing meat analogues or protein-rich snacks.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Human or animal studies This article does not contain any studies with human or animal subjects.

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Supplementary material

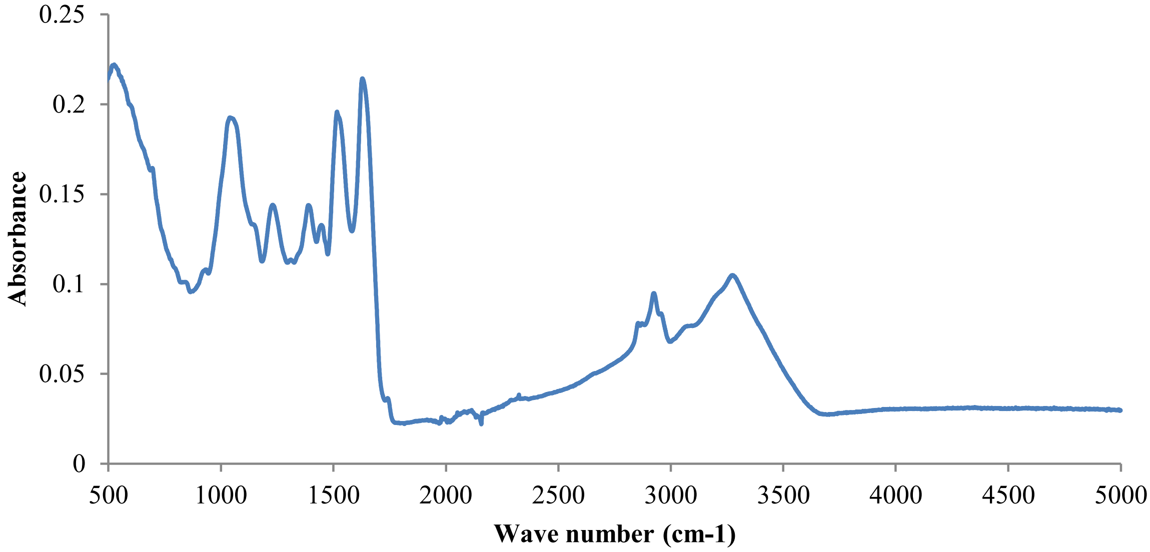

Fig.1. Original spectra of 30% spirulina added extrudate (trial number 3)

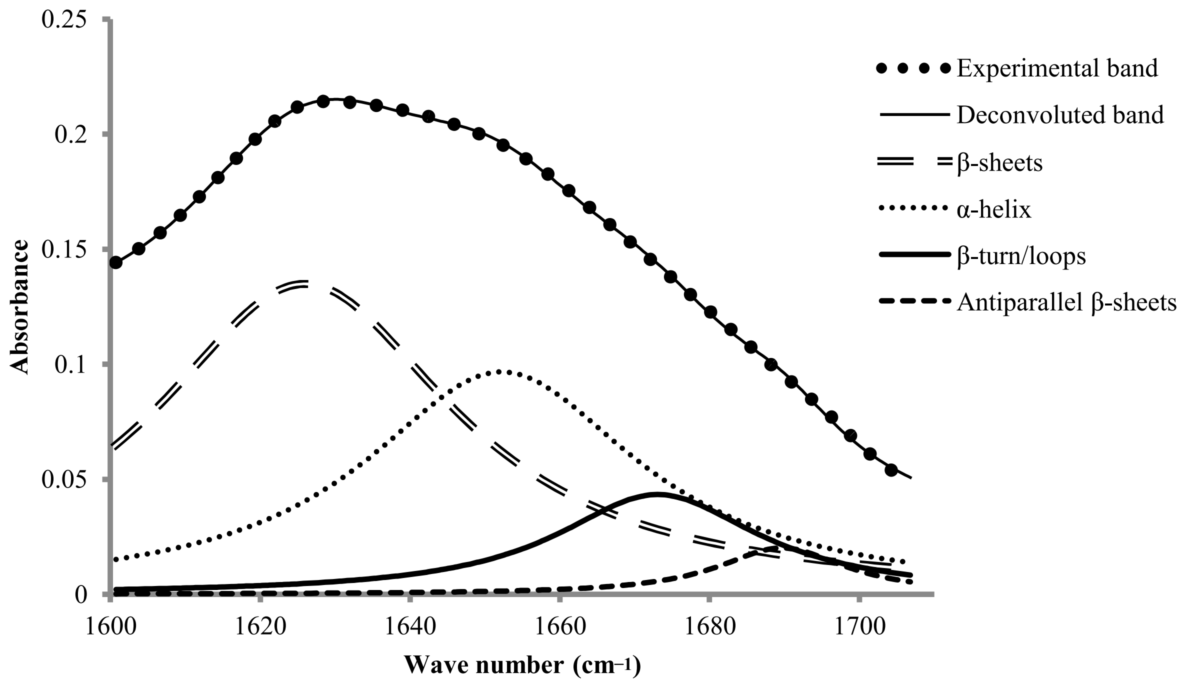

Fig. 2. Deconvoluted spectra of 30% spirulina added extrudate (trial number 3)

5. Publication: Influence of iota carrageenan addition on the properties of soya protein meat analogues

Preface

The study investigated the application of hydrocolloids with the focus on improving the texture of the soya meat analogues using a planetary roller extruder (PRE). Soya protein was chosen for this work, as it is a well-researched protein, and it would be a good starting point to use an additive and also to apply a new extrusion method. The use of additives for HME based meat analogues is still an unexplored area. As an additive, a food-grade hydrocolloid was chosen, as earlier studies showed that hydrocolloids are an effective texture modifier in protein based food products. The preliminary experiments with different types of carrageenans revealed that ICGN might have the potential as a texture modifier. Hence, different concentrations of ICGN were tested in order to find the optimum concentration for the texture improvement of soya meat analogues.

The objective of this work was to use different concentrations of ICGN and evaluate the physical, textural and sensory properties of the soya meat analogues.

In this study, Stefan Töpfl, Ralf G. Berger contributed the project ideas and were involved throughout the manuscript preparation process. Kemal Aganovic contributed to the manuscript by reading and correcting.

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Influence of iota carrageenan addition on the properties of soya protein meat analogues

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ABSTRACT

The aim of the study was to investigate the effect of addition of iota (ι) carrageenan (ICGN) on physical properties (cooking yield, expressible moisture, and colour), texture, sensory parameters and microstructure of soya meat analogues produced by high moisture extrusion processing. The high moisture extrusion trials were carried out using soya protein concentrate with the addition of 0.75%, 1.5%, 2.25% and 3% ICGN (by dry mass). The colour of the extrudates was not affected drastically by the addition of ICGN. Expressible moisture and cooking yield were decreased and textural properties, such as cutting force and elasticity, were increased significantly upon the addition of ICGN. Scanning electron microscopic observations showed that increasing ICGN levels led to a more compact network in the meat analogues supporting the changes obtained in texture, cooking yield, and expressible moisture. Sensory evaluation results confirmed that the increase in ICGN concentration led to harder, more fibrous and less juicy products resulting in a significantly improved overall acceptance. The extrudate with 1.5% ICGN was preferred by the panellists.

1. Introduction

Plant protein texturization is used to develop meat analogues from plant based proteins to replace animal proteins in the human diet. One of the ways to texturize the plant proteins is through extrusion cooking. The plant-based meat analogues are produced in a way to mimic some of the qualities of meat such as texture, flavour and appearance. Since 1960, low moisture extrusion technology (<35% moisture) has been used to produce traditional meat analogues which have a sponge like texture, and these products are supposed to be rehydrated before consumption (Guy, 2001). However, these products are not well comparable in terms of appearance and texture to the meat. In recent years, high moisture extrusion technology was used to produce meat analogues and considered as a promising technology to obtain fibrous meat-like structures from plant proteins. Texturization with high moisture extrusion is entirely different from other protein texturization processes (e.g., manufacturing of sausages, cheese curds, tofu, fibre formation by spinning or by extrusion cooking, etc.) (Cheftel, Kitagawa, & Quéguiner, 1992). During extrusion, proteins are plasticized in the heating chamber of an extruder and texturized in a long cooling die at the end of the extruder by varying the moisture, temperature, pressure and shear, respectively (Noguchi, 1990). These products are semi–finished and have to be post-processed before being served (e.g., further cooking, marinating as in the meat product preparations). Traditionally, a twin screw extruder is used for texturization. In this study, a planetary roller extruder (PRE) was used which produced less shear during extrusion compared to a twin screw extruder. The most common protein source used for the meat analogues production until to date is soya beans. Soya bean based ingredients, such as soya flour, protein concentrate and protein isolate, have been successfully used in the food industry for many years to develop meat analogues. Other plant protein sources considered for meat analogue production are wheat, cotton seed, legumes, lupine, pea etc. (Asgar, Fazilah, Huda, Bhat, & Karim, 2010).

One of the main problems considering the consumer acceptance of meat analogue products is texture. The texture may be modified or improved by adjusting the process conditions or by incorporating food additives. Polysaccharides are one of the main additives generally used in food industries for texture optimization. The effect of hydrocolloids on low moisture texturized soya protein was

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investigated by Boison, Taranto, and Cheryan (1983) and Berrington, Imeson, Ledward, Mitchell, and Smith (1984). To date, no studies were conducted on the effect of hydrocolloids on the soya protein texturization with high moisture extrusion.

In this study, iota (ι) carrageenan (ICGN) was chosen as an additive to incorporate during the extrusion process to improve the texture and functional properties of meat analogues. Carrageenans are sulfated anionic polysaccharides extracted from red algae. There are three main types of carrageenan called kappa (κ), iota (ι) and lambda (λ). They are differentiated based on the number and position of sulfate groups on the galactose/anhydrogalactose chain. κ-carrageenan contains one sulfate group, whereas ι and λ have two and three per disaccharide repeating unit, respectively (Imeson, 2000). Carrageenans are applied in food products as stabilizers, thickeners and gelling agents (Dickinson & Pawlowsky, 1997). Under certain conditions, κ-carrageenan and ICGN are able to form thermoreversible gels. In contrast, λ-carrageenan does not possess gelling capacity and is mainly used as thickening agent in foods (Yuguchi, Thu Thuy, Urakawa, & Kajiwara, 2002).

Various specific interactions, such as H-bonding, hydrophobic and steric interactions are responsible for the hydrocolloid-protein interactions (De Kruif & Tuinier, 2001). Sulfated polysaccharides form soluble complexes with proteins above their isoelectric pH (Kilara, 2005). Sulfate groups from the polysaccharides interact with positively charged sites available from protein moieties, such as ε-amino, α-amino, guanidinium, and imidazole. The intensity of the reaction varies depending on the number and distribution of these sites and overall charge of the protein.

In preliminary experiments, different types of carrageenans, such as κ-Carrageenan, sodium ICGN, calcium ICGN and λ-Carrageenan were used for the extrusion trials (results not shown). It was found that calcium ICGN had a positive influence on the texture. Hence, different percentages of ICGN were applied and the effects on the properties of soya meat analogues were investigated.

2. Materials and methods

2.1. Materials

Soya protein concentrate (ALPHA 8 IP) was purchased from Solae, LLC (St. Louis, Missouri, U.S.A.) and it contained 4.6% of moisture, 66.5% of protein and 2% of fat according to manufacturer's data. ICGN was obtained from Welding GmbH, Hamburg, Germany. According to manufacturer's information, ICGN contained 3% $CaCl_2$ as a residue from the manufacturing process.

2.2. High moisture extrusion cooking

The high moisture extrusion of soya protein concentrate and ICGN blends was carried out using a PRE (ENTEX Rust & Mitschke GmbH, Münster, Germany). The PRE is configured with a tempered jacket, rotating screws and an outlet die. The working mechanism of PRE is different compared to traditional single and twin screw extruders, as it works like a planetary gear. In twin screw extruders, mechanical energy dominates, whereas in PRE thermal energy is the dominant energy. An internal teethed pipe is connected to the jacket of the PRE. The long central screw is connected with the motor. When the extruder is operated, the central screw first starts to rotate and thereby initiating simultaneous the rotation of the planets. Thus, the material is conveyed by rolling up and drawn to the outlet die. The working principle of PRE is shown in Fig.1.

Length of the whole central pipe and teethed pipe is 1248.8 mm and 1348.5 mm respectively. Inside diameter of the outlet die is 14 mm. Inside the extruder there are three rotator parts, each with 400 mm length. In each of the rotators, six planets are integrated which are rotating the material inside, causing a thin layering of the material. Among the six planets, three are 399 mm and other 3 are 378 mm long.

At the end of the extruder, a cooling die was attached. Internal dimensions of the cooling die were 4.8 × 1.2 × 100 cm (W × H × L). ICGN was added with soy protein concentrate at the concentrations of 0.75%, 1.5%, 2.25% and 3% of dry mass, and the dry raw material blend was fed to the extruder at the rate of 6 kg/h. Water was fed at the rate of 10 kg/h. Temperature of zones 1 to 3 (60 °C, 135 °C, and 125 °C), cooling die (20 °C) and screw speed (50 rpm) were kept constant. A pressurized water unit system was used to heat the 3 extruder processing zones. The extrusion trials were performed in duplicates, and the samples were collected and stored at −20 °C until analyses.

2.3. Moisture content

The moisture content of the extruded meat analogue samples was analysed according to the AACC method (2000).

2.4. Physical properties

2.4.1. Cooking yield

The extruded samples were cut into 2 × 2 cm (L x W) cubes and cooked for 20 min in water at 80 °C. The mass before and after cooking was measured and cooking yield is calculated using equation (1).

Cooking yield (%) = (Mass of cooked sample/Mass of raw sample) × 100 (1)

2.4.2. Expressible moisture

The modified Hamm procedure (Grau & Hamm, 1957) was used to analyse the expressible moisture. Around 1 g of cooked meat analogue was placed between two filter papers and placed under a manual press weighing around 10 kg. The sample was subjected to pressure for 2 min.

Mass of the sample was taken before and after the pressing, and expressible moisture was expressed as a percentage of the net mass difference from the initial mass (equation (2)).

Expressible moisture (%) = (initial mass - squeezed mass) / (initial mass) × 100 (2)

2.4.3. Colour measurement

Colour measurements of the extruded samples and raw material mixtures were performed using spectrophotometer (CM-600, Konica Minolta Sensing Inc., Japan). The instrument records the L* (lightness), a* (green-red) and b* (blue-yellow) values. The measurements were taken at six different points on the surface of each of the samples. Total colour difference (ΔE) of the samples was calculated according to Altan, McCarthy, and Maskan (2008) using equation (3).

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (3)$$

The subscript 0 indicates the measurements of the raw material mixtures.

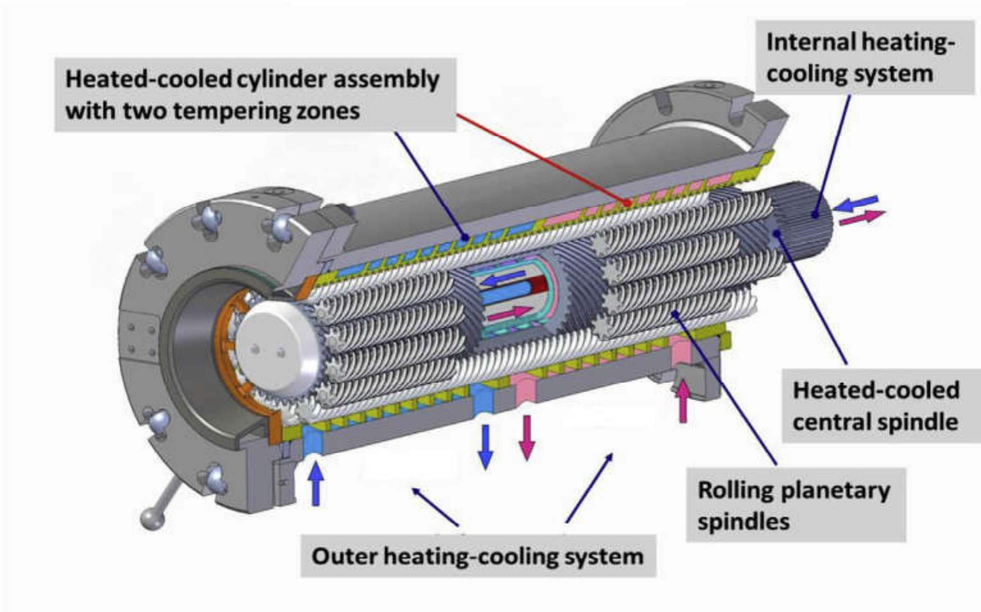

Fig. 1. Working principle of planetary roller extruder (PRE) (Source: ENTEX Rust & Mitschke GmbH).

2.5. Texture analysis

The textural properties, such as cutting force in longitudinal (L) and transverse (T) directions and elasticity, were measured using a texture analyser (Stable Micro Systems TA XT 2, Surrey England). The system was calibrated using a 5 kg cell. The square shaped samples (2 × 2 cm) were cut in L and T directions using a razor blade with a pre-test speed of 1 mm/s, a test speed of 2 mm/s and a post-test speed of 10 mm/s. The sample was also subjected to a compression test with a pre-test speed of 1 mm/s, a test speed of 0.5 mm/s and a post-test speed of 0.5 mm/s. The total test distance was 4 mm and the trigger value was 0.01 N. Five measurements were taken for each sample.

From the results of compression test, the elasticity of the samples was calculated using equation (4).

$$\text{Elasticity} = \Delta l / l_0 \tag{4}$$

Where Δl (reversible deformation distance) = total compressed distance (l_0) - non reversible distance (l).

2.6. Microscopy

2.6.1. Stereo microscopy

Stereo microscopy (Wild Heerbrugg, Switzerland) was used to interpret the laminar flow fibre layers formed in the samples. The samples were cut into small pieces and coated with green ink to distinguish the fibrous structure layers in the product. The light was passed from the bottom through the sample and the pictures were taken.

2.6.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to analyse the microstructure of the meat analogues. The samples were cut into pieces of 3 mm width and 10 mm length and subjected to flash freezing using liquid nitrogen slush and broken. Free water of the samples was removed at −8 °C under vacuum at 1×10^{-4} mbar for 4 h using an Emitech K1250 Cryo-SEM system. Then, the sample surfaces were sputter coated with gold and images were taken by Cryo- SEM (JEOL JSM- 6460 LV, Japan) at −180 °C.

2.7. Sensory evaluation

Sensory evaluation of the meat analogues was assessed using panellists in two sessions. In the first session, the samples from a first run were evaluated by 18 panellists, and in the second session 17 panellists assessed the second run samples. The panellists are involved in the sensory evaluation of foods regularly, but received no specific training for the extrudates. The samples were cut into 2 × 2 cm cubes and cooked for 20 min in water at 80 °C. After the cooking, the samples were cooled down at room temperature and served to the panellists. There were no seasonings and spices added to the samples to avoid any influence on the perception of texture. The panellists were asked to rank the firmness, juiciness, elasticity, fibrousness and over all acceptance of the products based on a 6 point hedonic scale (1 = extremely less firm, extremely less juicy, extremely less fibrous and extremely dislike, respectively; 6 = extremely more firm, extremely more juicy, extremely more fibrous and like extremely).

2.8. Statistical analysis

All the analyses were performed at least in triplicate with duplicate samples. One way Analysis of Variance (ANOVA) test was conducted to test the significance (significance level at $p < 0.05$) of the properties between the samples using the statistical programme DataStar 3.4.

3. Results and discussion

3.1. Extrusion processing

Processing conditions during the extrusion processing were noted (Table 1). There was an increase in pressure in zone 1 and 3 with increasing the concentration of carrageenan. This was attributed to the fact that ICGN increased the viscosity of the molten soya protein mass inside the extruder. Although the pressure increased, the torque values were found the same for the treatments with

Table 1
Response parameters for the extrusion of soya protein concentrate and ICGN blends.[a]

Samples	Pressure zone 1 (kPa)	Pressure zone 3 (end of processing zone) (kPa)	Torque (Nm)	Throughput (kg/h)	Moisture (%)[b](Mean ± Standard deviation)
0% ICGN	350	1050	20.5	16.15	60.23 ± 0.49
0.75% ICGN	600	1350	21.5	16.35	59.78 ± 0.37
1.5% ICGN	750	1550	21.5	16.25	60.16 ± 0.59
2.25% ICGN	950	1800	21.5	16.30	59.59 ± 0.30
3% ICGN	1250	2150	23.0	16.05	59.30 ± 0.52

[a] Mean values of two trials.
[b] Moisture content of the extrudates.

0.75%, 1.5% and 2.25% ICGN, but still higher than in the control sample. The sample with 3% ICGN had the highest torque value which was 23 Nm.

Berrington et al. (1984) studied the effect of alginate, guar gum, locust bean gum, carboxyl methyl cellulose, pectin and carrageenan with soya grits on the extrusion behaviour at low moisture content. Among the tested hydrocolloids, only alginate at 1% level (by dry mass) had an influence on extruder torque. The torque was decreased due to decreased viscosity which is in contrast to our present study. This could be due to the difference in moisture content and raw material between the above mentioned and our study. It can be assumed that the extruder responses may vary depending on the type of hydrocolloid, hydrocolloid concentration, type of raw material, extrusion conditions (for eg, temperature, moisture etc.) and type of extruder used.

3.2. Physical properties

3.2.1. Cooking yield

The cooking yield of the samples varied from 145.2% to 138.2% (Table 2). There was a significant difference found between control and samples with ICGN. It was observed that by increasing the ICGN concentration cooking yield decreased accordingly. This could be related to the lower water uptake during cooking of the samples containing ICGN. It can be assumed that the compact protein network created with carrageenan led to the lower water uptake. The free space for entering water into the protein network was reduced by increasing the concentration of carrageenan. Also, the weaker protein network created in the control sample could have allowed more penetration of water than the tight network created in ICGN added samples (Laleg, Cassan, Barron, Prabhasankar, & Micard, 2016). This was supported by the SEM images (section 3.4) which showed a more compact protein network in the carrageen containing samples than in the control.

3.2.2. Expressible moisture

Expressible moisture is one way of determining the water-holding capacity (WHC) of foods (Pein & Sherbon, 1979). Lower WHC indicates higher expressible moisture content and vice versa. The moisture loss of meat analogues as measured in the current study ranged from 16.59% to 8.60% (Table 2). The moisture loss was reduced while increasing the concentration of ICGN, thus improving the WHC of meat analogues. This could be due to the fact that ICGN trapped more water in the interstitial spaces of the gel (Wang, & Hesseltine, 1982). Since the protein network was also compact in ICGN added samples, the water release from the network after pressing was lower compared to the control sample. Thus, the firmness of the protein network influenced the WHC, since water was better retained in firm structure than in the soft gel network (Plock & Kessler, 1992).

There are numerous studies reporting that ICGN can improve the WHC in meat and meat based products. Ayadi, Kechaou, Makni, & Attia, 2009 reported that ICGN improved the WHC of turkey meat sausages. In a recent study (Shen & Kuo, 2017), it was shown that a κ-carrageenan/ICGN-mixture improved the WHC of tofu. The ICGN also improved the WHC of wheat flour (Martínez, Macias, Belorio, & Gómez, 2015), blue whiting muscle (Perez-Mateos & Montero, 2000), squid mantle gels (Gómez -Guillén & Montero, 1997) and Moo yor (thai sausage) (Nicomrat, Chanthachum, & Adulyatham, 2016).

3.2.3. Colour measurement

The colour measurements showed that the colour of the cooked extrudates was visually not much affected by the addition of ICGN, even though the statistical analysis showed differences among the samples (Table 2). This could be due to the fact that the amount of ICGN added was low. There were only slight variations observed in L*, a*, b*, and ΔE values between the control and samples with carrageenan. The difference in L and ΔE values should be above one unit in order to be differentiated by human eyes (Poynton, 1996), which was not the case in our study.

3.3. Texture analysis

In general, cooking reduced cutting force values (L and T) for all of the samples (Table 3). The results of the cutting force in L and T directions were positively correlated with the ICGN concentration. Cutting force can be interpreted as indirect measurement of hardness and texturization. The increase in hardness was probably due to the more dense network formation after ICGN addition. The

Table 2
Physical properties of meat analogues.

Samples	Cooking yield (%)	Expressible moisture (%)	Colour profile			
			L*	a*	b*	ΔE
0% ICGN	145.2 ± 1.54[a]	16.59 ± 1.14[a]	61.45 ± 0.32[a]	2.71 ± 0.08[d]	11.55 ± 0.22[a]	27.80 ± 0.32[b]
0.75% ICGN	142.6 ± 1.88[b]	16.15 ± 1.22[a]	60.72 ± 0.56[b]	3.10 ± 0.18[b]	11.75 ± 0.45[ab]	28.31 ± 0.56[a]
1.5% ICGN	141.5 ± 1.62[bc]	14.41 ± 0.46[b]	61.28 ± 0.63[a]	2.91 ± 0.11[c]	12.05 ± 0.18[a]	27.76 ± 0.67[b]
2.25% ICGN	139.9 ± 1.05[c]	11.57 ± 1.10[c]	61.55 ± 0.36[a]	3.38 ± 0.11[a]	12.08 ± 0.35[a]	27.50 ± 0.46[b]
3% ICGN	138.2 ± 0.96[d]	8.60 ± 1.58[d]	61.20 ± 0.64[ab]	3.33 ± 0.20[a]	11.84 ± 0.60[ab]	27.70 ± 0.70[b]

Values are indicated as mean ± standard deviation.
Means within columns with different superscripts are significantly different ($p < 0.05$).

Table 3
Textural properties of meat analogues.

Samples	Raw			Cooked		
	Cutting force- L (N)	Cutting force- T (N)	Elasticity	Cutting force- L (N)	Cutting force- T (N)	Elasticity
0% ICGN	7.55 ± 0.45^{c}	7.85 ± 0.14^{c}	0.45 ± 0.09^{c}	4.51 ± 0.23^{d}	3.69 ± 0.26^{d}	0.45 ± 0.15^{b}
0.75% ICGN	8.08 ± 0.78^{bc}	9.17 ± 0.32^{b}	0.53 ± 0.05^{b}	5.70 ± 0.49^{c}	4.99 ± 0.43^{c}	0.58 ± 0.08^{a}
1.5% ICGN	8.11 ± 0.62^{b}	9.26 ± 0.54^{b}	0.57 ± 0.05^{b}	6.31 ± 0.69^{b}	6.98 ± 0.21^{b}	0.56 ± 0.11^{ab}
2.25% ICGN	8.30 ± 0.72^{ab}	10.05 ± 0.45^{a}	0.61 ± 0.04^{a}	6.61 ± 0.64^{b}	7.10 ± 0.30^{b}	0.62 ± 0.06^{a}
3% ICGN	8.86 ± 0.81^{a}	10.39 ± 0.32^{a}	0.56 ± 0.06^{b}	7.29 ± 0.74^{a}	7.60 ± 0.25^{a}	0.58 ± 0.09^{a}

Values are indicated as mean ± standard deviation.
Means within columns with different superscripts are significantly different ($p < 0.05$).

presence of ICGN also influenced the gelling process of protein, thus modified the texture (Ayadi et al., 2009). ICGN was found to increase the hardness in tofu (Shen & Kuo, 2017), turkey sausages (Ayadi et al., 2009) and Moo yor (Nicomrat et al., 2016). In low moisture (20%) extruded corn product, carrageenan increased the breaking strength (Maga & Fapojuwo, 1988).

The both raw and cooked sample with 2.25% ICGN had highest elasticity and it was significantly different from the sample without ICGN (Table 3). This can be explained due to the differences in intermolecular structure of the samples (Fig. 3). When increasing ICGN concentration up to 2.25%, the structure becomes more compact and the open space (holes) between the network structures becomes smaller and numerous. It is assumed that upon compression test, the loose network structure with larger spaces in the sample without ICGN might easily be deformed than the more closed network structure with many smaller spaces in the sample with 2.25% ICGN. The elasticity differences between the raw samples containing 0.75%, 1.5% and 3% ICGN are not significant, while no significant differences between all ICGN added cooked samples could be detected. In the sample containing 3% ICGN, hardly any network and open spaces between the elements were found and it appeared more like a block. It should also be noticed that this sample had the highest cutting strength. It can be assumed that the firm structure without any spaces is likely to get destroyed upon compression. Thus the product cannot have more reversible distance resulting in a reduction of elasticity compared to the sample with 2.25% ICGN.

3.4. Microscopical observations

Stereo microscopic pictures showed a laminar flow structure within the products (Fig. 2). It was visible from the pictures that the layers and the products became thicker with increasing concentration of ICGN. In the control sample, the layers appeared like broken, whereas in ICGN added samples, thick uniform layers were found. As discussed in the earlier section, increasing ICGN concentration up to 2.25% led to more compactness in the network structure of the extrudate (Fig. 3). The sample with 3% ICGN had huge difference and no network structure was found compared to all the other samples. The images obtained by SEM support the results of the texture analysis (section 3.3) and sensory evaluation (section 3.5): the denser the microstructure the harder the sample, and for juiciness vice versa. The microstructural changes in the ICGN added samples could be related to the interactions between ICGN and soya protein. In an extrusion process, proteins are denatured due to heat and shear actions. During the denaturation of proteins, the buried reactive sites become exposed and the structure of the protein turns into a “random coil” state which is flexible, allows configurational adjustments and favours the protein-polysaccharide interactions (Ledward, 1994). Three types of interactions such as weak or strong, specific or nonspecific, attractive or repulsive are responsible for the complex formation between protein-polysaccharides (Dickinson, 1994). However, the amount of contribution of each type of interaction for the microstructural and textural properties of meat analogues under extrusion conditions remains unclear.

3.5. Sensory evaluation

The sensory evaluation (Table 4) indicated that the increase in concentration of ICGN resulted in increased firmness which correlated with the instrumental analysis (section 3.3). The juiciness of the products was negatively correlated with the firmness which was also in correlation with cooking yield results meaning that the firmer the product the less water uptake and juiciness. One of the main factors considered when consuming the meat alternatives is a mouth feel and fibrousness similar to meat. The increase in ICGN concentration led to an increase in fibrousness of the product. This could be due to the denser structure, and also the firmness of the product created the feeling of more fibrousness in the mouth. As observed in instrumental analysis, increased elasticity could be detected up to 2.25% ICGN and at 3% ICGN elasticity started decreasing significantly. The scores for the overall acceptance ranged from 1.73 to 2.49. The reason for the general lower acceptance scores can be related to the tasting of plain cooked intermediate product without any seasonings and spices. However, the acceptability can be certainly further improved in case of end products e.g. nuggets- or burger like products with seasonings and spices. The sample with 1.5% ICGN received the highest score and was significantly different compared to control sample. At ICGN concentrations of 2.25 and 3%, the acceptance started to decrease, but was still higher than the control. Even though the fibrousness was positively correlated with the ICGN concentration, the factor hardness also could have influenced the preference. The samples with 2.25 and 3% ICGN were harder, thus these samples were scored lower compared to 1.5% ICGN.

4. Conclusion

The influence of ICGN addition on the properties of soya meat analogue was studied. The addition of ICGN had a positive impact on the quality characteristics, such as expressible moisture, texture and sensory, except cooking yield. Soya meat analogue can be formulated with 1.5% ICGN to obtain better physical, textural and sensorial properties. Furthermore, the study confirmed that a low shear extrusion process was suitable for the texturization of proteins. Thermal energy appeared to play a bigger role in protein texturization than mechanical energy. Future studies should focus on the molecular interaction of protein and hydrocolloids under extrusion conditions in order to understand and tailor the texture formation of extrusion based food products.

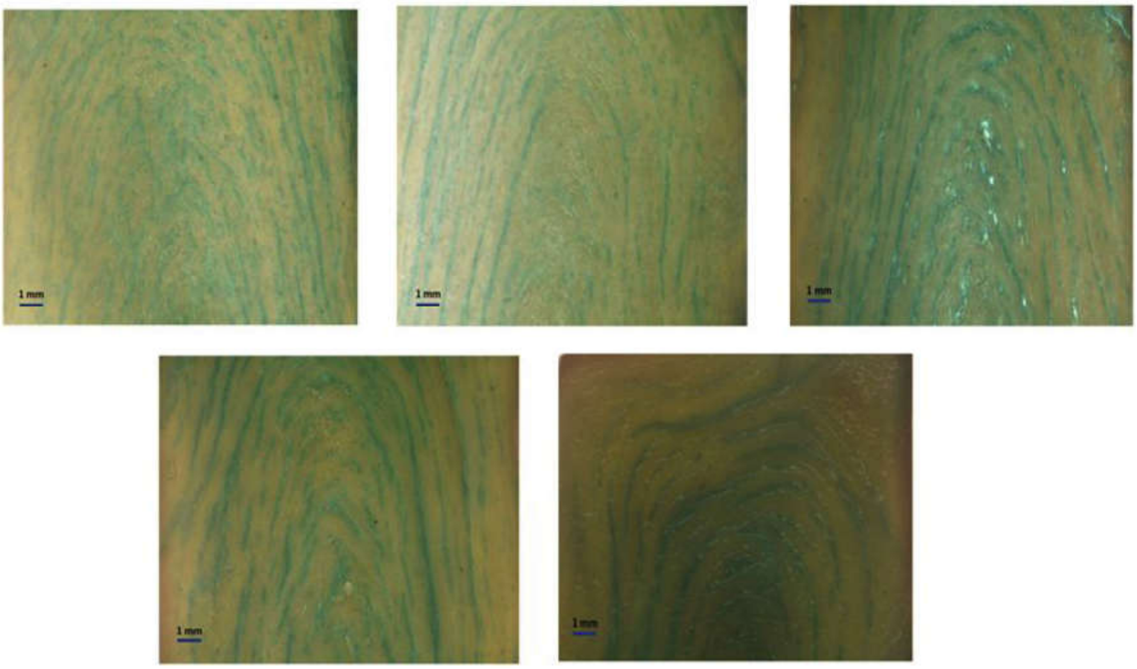

Fig. 2. Laminar flow profile of meat analogues (from left to right: 0%, 0.75%, 1.5%, 2.25% and 3% carrageenan added samples).

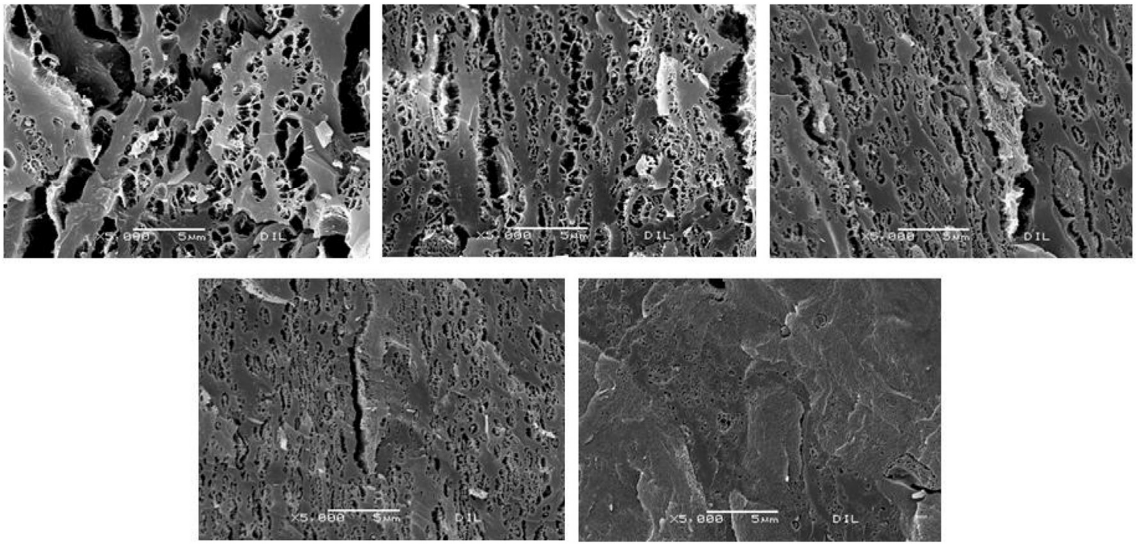

Fig. 3. SEM images of meat analogues (from left to right: 0%, 0.75%, 1.5%, 2.25% and 3% carrageenan added samples). Scale bars indicate 5 μm.

Table 4
Sensory mean scores of meat analogues.

Samples	Firmness	Juiciness	Fibrousness	Elasticity	Over all acceptance
0% ICGN	1.94 ± 1.14^{c}	3.45 ± 1.32^{a}	1.82 ± 1.36^{b}	2.17 ± 1.47^{b}	1.73 ± 1.12^{b}
0.75% ICGN	2.70 ± 1.32^{b}	2.74 ± 1.28^{b}	1.98 ± 0.99^{b}	2.49 ± 1.32^{ab}	1.97 ± 1.10^{ab}
1.5% ICGN	2.81 ± 1.16^{b}	2.66 ± 1.37^{bc}	3.09 ± 1.26^{a}	2.98 ± 1.13^{a}	2.49 ± 1.28^{a}
2.25% ICGN	3.32 ± 1.39^{ab}	2.62 ± 1.15^{bc}	3.43 ± 1.34^{a}	2.73 ± 1.17^{a}	2.35 ± 1.34^{a}
3% ICGN	3.77 ± 1.36^{a}	2.11 ± 1.23^{c}	3.51 ± 1.24^{a}	2.18 ± 0.86^{b}	2.17 ± 1.05^{ab}

Values are indicated as mean ± standard deviation.
Means within columns with different superscripts are significantly different ($p < 0.05$).

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6. Outlook

The research confirmed the feasibility of lupin protein and partial utilization of *Spirulina* biomass for the development of meat analogues using HME. The use of *Spirulina* biomass enriched the antioxidant properties of lupin meat analogues. Further it was proven that a low shear extrusion method was possible for the meat analogues production. It was also further shown that the texture of the meat analogues could be improved by the addition of ICGN. However, in the future further studies could be conducted on the following aspects.

The plant based protein concentrates/isolates used in the production of meat analogues are often deficient/lacking in some of the nutrients such as iron, zinc, vitamin B_{12}, vitamin D_3 and docosahexaenoic acid, which are found abundantly in meat and animal based products. Hence, the nutritional composition can be further improved in the meat analogues by the addition of nutrients during extrusion. The effect of extrusion on the retention and functionality of nutrients in the meat analogues can be further assessed.

The *Spirulina* biomass used in the study resulted in dark green coloured extrudates, which could be avoided by extraction of the pigments from the *Spirulina*. On the other hand, red coloured microalgae, for example *Haematococcus sp.* or other heterotrophic microalgae, can be used to mimic the colour qualities of the meat. Furthermore, the addition of natural food colours and their stability during storage can be studied.

The improved IVPD and antioxidant properties of the protein ingredients after HME must be backed up by conducting *in-vivo* studies to confirm the bioavailability of antioxidants and protein digestibility.

The planetary roller extruder can be further investigated for other protein sources as well. Further investigations on understanding the molecular mechanism of hydrocolloid and protein interaction can be conducted for the meat analogues. Other types of food additives can also be explored for the improvement of texturization. Thus, the opportunities for exploring and researching the plant based proteins are endless to create sustainable meat analogues.

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8. Curriculum Vitae

Personal Information

Name:	Megala Palanisamy
Date of Birth:	15.07.1986
Place of Birth:	Salem, India
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Education

Since 05/2015	**PhD student** **University of Hannover**, Institute of Food chemistry, Germany PhD topic: High moisture extrusion of plant proteins
09/2010 - 03/2013	**Master of Science in Food Technology** **Wageningen University and Research Centre**, The Netherlands Area of Specialization: Dairy Science and Technology Thesis topic: The influence of free fatty acids on lipase activity of raw milk
10/2007 - 10/2009	**Master of science in Food and Nutrition** (University Topper) **Acharya N. G. Ranga Agricultural University**, Hyderabad, India Area of Specialization: Nutrition and Dietetics Thesis topic: Development and evaluation of Inulin and Fructooligosaccharides incorporated fruits based functional foods
07/2003 - 04/2007	**Bachelor of Science in Home Science** **Tamil Nadu Agricultural University**, Coimbatore, India Area of Specialization: Food Technology Thesis topic: Development of dietary fiber rich health mix

Work Experience

Since 09/2018	**Raw material and process specialist** Nordgetreide GmbH & Co. KG Lübeck, Germany
05/2015 -08/2018	**Research Associate** German Institute of Food Technologies Quakenbrück, Germany

Responsibilities:
- Worked in an interdisciplinary research project focusing on sustainability transitions in food production
  Project website: https://www.uni-goettingen.de/de/519937.html
- Developing meat analogues from plant based proteins and alternative proteins like algae with extrusion technology
- Optimizing the extrusion process and physico-chemical analysis of meat alternatives (e.g., texture and structure analysis, antioxidant activities, protein digestibility, protein structure characterization etc.)
- Project management

11/2013 - 06/2014	**Intern** Fraunhofer Institute for Process Engineering and Packaging Freising, Germany
03/2012 - 02/2013	**R&D Trainee** Friesland Campina, Wageningen, The Netherlands
10/2009 - 07/2010	**Research Associate** Acharya N. G. Ranga Agricultural University Hyderabad, India

Fellowships

01/2012 - 02/2013	Food Valley Ambassador Fellowship Sponsor: Food Valley Organization, The Netherlands
09/2010 - 08/2012	Anne van den Ban Fellowship and Dairy science and technology Fellowship Sponsors: Anne van den Ban foundation and Wageningen University
10/2007 - 10/2009	Junior Research Fellowship Sponsor: Indian Council of Agricultural Research

Publications

- **Palanisamy M,** Töpfl S, Berger RG, Hertel C (2019) Physico-chemical and nutritional properties of meat analogues based on Spirulina/lupin protein mixtures. Eur Food Res Technol. doi: https://doi.org/10.1007/s00217-019-03298-w

- **Palanisamy M,** Franke K, Berger RG, Heinz V, Töpfl S (2019) High moisture extrusion of lupin protein: influence of extrusion parameters on extruder responses and product properties. J Sci Food Agric. 99: 2175–2185. doi: 10.1002/jsfa.9410

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- **P. Megala** and T.V. Hymavathi, 2011.Inulin and Fructooligosaccharides Incorporated Functional Fruit Bars, World Academy of Science, Engineering and Technology Vol 59, pp 600-605.